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MONOCLONAL-%ANTIBODY% TO HUMAN C-%MPL% RECEPTOR STIMULATES IN-VITRO MEGAKARYOCYTOPOIESIS
Author(s): DENG B; WANG JF; BANU N; CAVACINI L; GROOPMAN JE; AVRAHAM H Corporate Source: HARVARD UNIV,NEW ENGLAND DEACONESS HOSP,SCH MED,DIV HEMATOL ONCOL/BOSTON//MA/02215
Journal: EXPERIMENTAL HEMATOLOGY, 1996, V24, N9 (AUG), P260 ISSN: 0301-472X
Language: ENGLISH Document Type: MEETING ABSTRACT

Simultaneous activation of signals through gp130, c-kit, and interleukin-3 receptor promotes a trilineage blood cell production in the absence of terminally acting lineage-specific factors.
Kimura T; Sakabe H; Tanimukai S; Abe T; Urata Y; Yasukawa K; Okano A; Taga T; Sugiyama H; Kishimoto T; Sonoda Y
Department of Hygiene, Kyoto Prefectural University of Medicine, Kyoto, Japan.
Blood (UNITED STATES) Dec 15 %1997%, 90 (12) p4767-78, ISSN 0006-4971 Journal Code: A8G
Languages: ENGLISH
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We assessed the biologic role of signaling through gp130, a signal-transducing receptor (R) component, in human hematopoiesis in vitro. Although peripheral blood-derived CD34(+) cells ubiquitously expressed gp130 and interleukin-3 receptor alpha (IL-3Ralpha), IL-6Ralpha was only detected on 80% of these CD34(+) cells. We sorted CD34(+)IL-6R+ or
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The %Mpl% receptor is expressed in the megakaryocytic lineage from late progenitors to platelets.
Debili N; Wendling F; Cosman D; Titeux M; Florindo C; Dusanter-Fourt I; Schooley K; Methia N; Charon M; Nador R; et al
INSERM U362, Institut Gustave Roussy, Villejuif, France. Blood (UNITED STATES) Jan 15 %1995%, 85 (2) p391-401, ISSN 0006-4971 Journal Code: A8G
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The %Mpl% receptor (%Mpl% -R) is a cytokine
AB, NOT AGONIST

Author(s): Ohashi H; Morita H; Tahara T; Tsunakawa H; Matsumoto A; Ogami K; Oda A; Ikeda Y; Miyazaki H; Kato T
Corporate Source: KEIO UNIV,DEPT INTERNAL MED, DIV HEMATOL/TOKYO//JAPAN/; KIRIN BREWERY CO LTD,PHARMACEUT RES LAB/GUNMA//JAPAN/
Journal: BLOOD, %1997%, V90, N10,2,1 (NOV 15), P3533-3533
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References: 32 Title: Thrombin cleaves recombinant human thrombopoietin: One of the proteolytic events that generates truncated forms of thrombopoietin Author(s): Kato T (REPRINT) ; Oda A; Inagaki Y; Ohashi H; Matsumoto A; Ozaki K; Miyakawa Y; Watarai H; Fuju K; Kokubo A; Kadoya T; Ikeda Y; Miyazaki H
Corporate Source: KIRIN BREWERY CO LTD,PHARMACEUT RES LAB, 3 MIYAHARA CHO/TAKASAKI/GUMMA 37012/JAPAN/ (REPRINT); KEIO UNIV,DEPT INTERNAL MED, DIV HEMATOL, SHINJUKU KU/TOKYO 160//JAPAN/
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, %1997%, V94, N9 (APR 29), P4669-4674 ISSN: 0027-8424 Publication date: 19970429
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418
Language: English Document Type: ARTICLE
Abstract: A heterogeneity in the molecular weight (M-r) of thrombopoietin (TPO) has been reported, We found several

thrombin cleavage sites in human, rat, murine, and canine TPOs, and also found that human TPO

FILE:TPO PROCESSING

Title: A single injection of pegylated murine megakaryocyte growth and development factor (MGDF) into mice is sufficient to produce a profound stimulation of megakaryocyte frequency, size, and ploidization Author(s): Arnold JT; Daw NC; Stenberg PE; Jayawardene D; Srivastava DK; Jackson CW (REPRINT)
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Journal: BLOOD, %1997%, V89, N3 (FEB 1), P823-833
ISSN: 0006-4971 Publication date: 19970201
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399
Language: English Document Type: ARTICLE
Abstract: Despite numerous studies investigating the action of c-%mpl% ligand, no reports have defined the in vivo changes

FILE:TPO ADMINISTRATION

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MONOCLONAL-%ANTIBODY% TO HUMAN C-%MPL% RECEPTOR STIMULATES IN-VITRO MEGAKARYOCYTOPOIESIS
Author(s): DENG B; WANG JF; BANU N; CAVACINI L; GROOPMAN JE; AVRAHAM H Corporate Source: HARVARD UNIV,NEW ENGLAND DEACONESS HOSP,SCH MED,DIV HEMATOL ONCOL/BOSTON//MA/02215
Journal: EXPERIMENTAL HEMATOLOGY, 1996, V24, N9 (AUG), P260 ISSN: 0301-472X
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ABSTRACTS

259

AN ENGINEERED NOVEL FACTOR FOR EXPANSION AND DEVELOPMENT OF PLATELET PRODUCING CELLS. J.G. Giri*, L.E. Kahn*, N. R. Staten*, D. C. Wood*, J. P. Favara*, A..L. Abegg*, L. E. Pegg*, J. W. Thomas*, M. S. Huynh*, J. Monahan*, A. Farese*, T. MacVittie, C. M. Baum, L. W. Blystone* and J. P. McKearn*. Searle, Inflammatory Diseases Research, 700 Chesterfield Parkway N. St. Louis, MO 63198 and University of Maryland Cancer Center, Baltimore, MD 21201.

We have engineered a chimeric recombinant protein Promegapoietin (PMP), that promotes differentiation of platelet producing cells and expansion of their progenitors. Promegapoietin can bind and activate the c-mpl receptor and stimulate proliferation of c-mpl expressing cells. PMP stimulates multilineage expansion of CD34+ bone marrow cultures in vitro, in addition to the CD41+ megakaryocyte (MK) population. Differences in the nature of the response to c-mpl ligand compared to Promegapoietin were evident in the lineage subsets and maturational stage of the colonies observed in vitro. The most pronounced difference was the specific increase in the number of early BFU-MK colonies induced by PMP. Promegapoietin was shown to stimulate platelet production in C57BL/6 mice and to enhance platelet recovery in irradiated mice. Promegapoietin is effective in eliminating the thrombocytopenic nadir and stimulates rapid recovery of platelets in a rhesus radiation induced myelosupression model. Based on these results Promegapoietin appears to have promising applications as a platelet restorative agent.

260

AN AGONIST MURINE MONOCLONAL ANTIBODY TO THE HUMAN C-MPL RECEPTOR STIMULATES *IN VITRO* MEGAKARYOCYTOPOIESIS. B. Deng*, J.F. Wang*, N. Banu*, L. Cavacini*, J.F. Groopman*, H. Avraham* (Intro. by N.G. Abraham) Division of Hematology/Oncology, Deaconess Hospital, Harvard Medical School, Boston, MA, USA.

Thrombopoietin (TPO) is a hematopoietic growth factor that stimulates megakaryocytopoiesis and platelet production *in vivo* and promotes development of identifiable megakaryocytes *in vitro*. We have developed a murine monoclonal antibody BAH-1 raised against human megakaryocytic cells which specifically recognizes the c-Mpl receptor and demonstrates agonist activity by stimulating megakaryocytopoiesis *in vitro*. BAH-1 monoclonal antibody alone supported the formation of CFU-MK colonies similar to TPO. The combination of IL-3 plus BAH-1 or of BAH-1 plus hTPO significantly increased the number of human CFU-MK colonies. In addition, BAH-1 monoclonal antibody stimulated the proliferation and maturation of primary bone marrow megakaryocytes in a dynamic heterogeneous liquid culture system. Individual large megakaryocytes as well as small megakaryocytic cells were observed in cultures of $CD34^+$ $CD41^+$ cells in the presence of BAH-1 antibodies. Murine immature megakaryocytes showed a significant response to BAH-1 antibody with an increase in detectable numbers of acetylcholinesterase-positive megakaryocytes using the single cell growth assay, similar to TPO. However, BAH-1 failed to stimulate murine CFU-MK colonies. No effects of BAH-1 antibody were observed on CFU-GM, BFU-E or CFU-E colonies. This antibody should prove useful in mapping studies of the c-Mpl receptor, as well as evaluating the effects of sustained activation of the receptor in preclinical models of severe thrombocytopenia.

261

EFFECTS OF THROMBOPOIETIN AND APLASTIC SERUM ON HUMAN MEGAKARYOCYTE PROLIFERATION AND ENDOREPLICATION

X. Qiao*, M. Loudoveris*, C. Berger*, D. Van Epps, and J. Bender
Baxter Healthcare Corporation, Immunotherapy Division, Round Lake, IL

The effect of thrombopoietin (TPO, Genzyme) and aplastic serum on *in vitro* human *megakaryocyte (MK) production* was evaluated in cultures of cord blood mononuclear cells and selected CD34 cells from G-CSF mobilized normal donor apheresis products. Cultures were analyzed for cell expression of platelet glycoprotein IIb/IIIa (CD41a), MK ploidy and MK colony forming units (CFU-MK) using immunocytochemistry, flow cytometry and a slide colony assay, respectively. CD34 cell isolation from apheresis products was accomplished using the Isolex® 300 CD34 cell selection device. The selection resulted in a 4- fold enrichment of CFU-MK, a 2- fold enrichment of non-colony forming MK, and a 21- fold enrichment of granulocyte-macrophage colony forming cells. Cells from the same individual were grown in the X-VIVO serum-free medium supplemented with TPO alone, aplastic serum alone, and a cytokine mixture (stem cell factor, IL-3 and IL-6) with or without TPO/aplastic serum. The CFU-MK number peaked in 5 - 7 day cultures and increased 4 - 5 fold. Most cells had lost their MK colony forming ability in 10 - 12 day cultures. CD34 cell cultures supplemented with TPO-alone yielded the highest percentage of MK (93.8 ± 1.7%), while cultures supplemented with aplastic serum-alone yielded 17.2 ± 5.1% MK. About 40% and 10% of MK showed ≥ 8N and ≥ 16N DNA ploidy, respectively. However, the total number of MK in these cultures was low due to little or no proliferation (0.8 - 3 fold cell expansion). In comparison, cultures supplemented with the cytokine mixtures yielded a 61.7 ± 14.5 fold cell expansion and 13.5 ± 3.6% MK, demonstrating a 13 fold higher total MK number. The majority of MK (87.5 ± 5.0%) contained low ploidy (2N - 4N). Addition of aplastic serum to the cytokines nearly doubled the cell count without changing the MK percentage; while the addition of TPO nearly doubled the MK percentage without changing the cell count. Either addition further increased the total MK number 1.6 - 2 fold as compared to the cytokines without TPO or aplastic serum, but did not substantially increase the MK DNA ploidy. In conclusion, TPO or aplastic serum stimulates a high frequency and ploidy of MK, while a combination of TPO or aplastic serum with stem cell factor, IL-3 and IL-6 increases MK cell growth, but such combinations are less effective on the production of high ploidy MK.

262

GENERATION AND PLOIDY DEVELOPMENT OF MURINE MEGAKARYOCYTES IN RELATION TO CULTURE TIME AND CYTOKINE COMBINATIONS. Adrie Boudewijn*, Ruben de Kuiper*, Rosita Vossen* and Rob E. Ploemacher. (Intro. by Dimitri A. Breems). Department of Hematology, Erasmus University Rotterdam, 3000 DR, The Netherlands.

We have studied the contribution of IL-3, G-CSF, GM-CSF, SCF, IL-6, IL-11, IL-12, TPO and EPO on the generation and ploidyzation of Megakaryocytes (Meg) in serum-free liquid cultures of low density bone marrow cells from day-6 post 5-fluorouracil treated mice. The percentage of Megs generated in these cultures was 0-25 percent, and that upto $3x10^5$ Megs (ploidy 2-64 N) were formed in 7-11 days starting from $2x10^4$ diploid cells. The kinetics of Meg formation appeared highly dependent on the cytokine/chemokine combinations used as evidenced by monitoring the cultures between day 5 and 11. TPO (+/- EPO) generated few Megs while it showed little synergy with IL-11, IL-12 or IL-3 to increase Meg number and ploidy. However, TPO displayed extensive synergy with SCF, with combinations of SCF and IL-11, IL-12 or IL-3, or combinations of IL-3 and IL-11 or IL-12. TPO could not further increase Meg numbers and ploidy in the presence of IL-3+SCF+IL-11 (or IL-12). Irrespectively of the presence of other cytokines, IL-3 led to rapid Meg fragmentation with lower ploidy, even in subliminal concentrations, but total Meg numbers over the whole culture period were similar as in the presence of SCF. We found that particular cytokine combinations had differential effects on the Meg generation from cells sorted on the basis of expression of the Meg antigen 4A5. In this respect, SCF+IL-12 (+IL-3) stimulated high ploidy Megs from 4A5neg cells, but not as many from 4A5pos cells, while IL-3+IL-11+TPO+EPO generated many Megs from the 4A5pos, but not the 4A5neg, fraction. MRP-1 and TGF-B1 inhibited nucleated cell, Meg and CFU-C generation in SCF+IL-12+TPO+EPO stimulated cultures while the highest ploidy Megs were the most affected. IL-3 could partly abrogate these inhibitory effects. Significantly, this study shows that the kinetics of Meg generation *in vitro* are complex and result from (a) different cytokine requirements of Meg precursors and stem cell subsets, and (b) the loss of Meg number and ploidy by platelet formation at various developmental stages and culture time.

263

PRINCIPLES OF THROMBOPOIETIN PHYSIOLOGY: APPLICATION TO DISORDERS OF ALTERED PLATELET PRODUCTION. D. Kuter, Y. Xia*, Chun Yang*, Junzhi Li*. Massachusetts General Hospital, Boston, MA and Harvard Medical School, Boston, MA, USA.

Thrombopoietin (TPO) has recently been identified as the physiological regulator of platelet production. Studies in animals have identified a number of important physiological attributes of TPO: (1) TPO takes up to 24 hours to rise maximally after the onset of acute thrombocytopenia; (2) Circulating levels of TPO are inversely proportional to the platelet count in animal models of acute or amegakaryocytic thrombocytopenia; (3) TPO levels rise exponentially as the platelet count declines linearly in models of acute or amegkaryocytic thrombocytopenia; (4) Native TPO has a circulating half-life of under 45 minutes; (5) Platelets bind and clear TPO from the circulation; (6) Hepatic TPO production is not altered by changes in the platelet mass. These principles give rise to a model in which the TPO produced constitutively by the liver enters the circulation, is cleared to a variable extent depending on the number/function of the platelet mass and then modulates megakaryocyte growth.

In pathological conditions of platelet production this model provides some insights: (1) TPO levels are inversely proportional to the rate of platelet production in the steady state; (2) The level of constitutive TPO production may be altered by pathological disorders or other molecules; (3) The effect of TPO may be altered by pathological changes in its megakaryocyte receptor or post-receptor signalling events; (4) TPO clearance may be altered by pathological disorders or other molecules.

264

THE DELAYED PLATELET RECOVERY AFTER ALLOGENEIC CORD BLOOD TRANSPLANTATION COULD BE DUE TO AN INADEQUATE NUMBER OF CD34+CD41+ CELLS IN ALLOGRAFTS.

H.T.Hassan, Ph.Beremaan*, B.Schleimer*, A.R.Zander. Bone Marrow Transplantation Center, Hamburg University Hospital Eppendorf, Germany.

During the last five years, cord blood has been increasingly used for matched related allogeneic transplantation in children and young adults. However, delayed platelet engraftment was common. The present study was performed to investigate the possible reason(s) for the delayed platelet recovery observed after allogeneic cord blood transplantation by comparing the number of early CD34+CD41+ and late CD41+CD14- megakaryocytic progenitor cells in cord blood [n = 7] with those of bone marrow [n = 6] from healthy donors. The present results revealed that whereas the number of late CD41+CD14- megakaryocytic cells per ml. was significantly more in cord blood than in bone marrow, the number of early megakaryocytic CD34+CD41+ cells per ml. was less in cord blood than in bone marrow.

Median (Range) $x10^3$/ml	$CD41^+CD14^-$ Cells	$CD\ 34^+CD41^+$ Cells	
Bone Marrow	252.7 (66.4-622.2)	10.7 (3.1-14.6)	<0.01
Cord Blood	423.0 (81.9-635.8)	6.8 (6.0-17.6)	

We conclude that the delayed platelet recovery after allogeneic CB transplantation could be due to an inadequate number of CD34+CD41+ megakaryocytic progenitor cells in the CB allografts. Therefore, an efficient ex-vivo expansion of cord blood megakaryocytic progenitor cells using cytokines including thrombopoietin seems to be essential to achieve a more rapid platelet recovery after an allogeneic cord blood transplantation (An average 1 liter bone marrow harvest contains 10 times more CD34+ cells than an average 100 ml. cord blood harvest).

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Simultaneous activation of signals through gp130, c-kit, and interleukin-3 receptor promotes a trilineage blood cell production in the absence of terminally acting lineage-specific factors.

Kimura T; Sakabe H; Tanimukai S; Abe T; Urata Y; Yasukawa K; Okano A; Taga T; Sugiyama H; Kishimoto T; Sonoda Y

Department of Hygiene, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Blood (UNITED STATES) Dec 15 %1997%, 90 (12) p4767-78, ISSN 0006-4971 Journal Code: A8G

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Simultaneous Activation of Signals Through gp130, c-kit, and Interleukin-3 Receptor Promotes a Trilineage Blood Cell Production in the Absence of Terminally Acting Lineage-Specific Factors

By Takafumi Kimura, Hideaki Sakabe, Shigeatsu Tanimukai, Tatsuo Abe, Yoji Urata, Kiyoshi Yasukawa, Akira Okano, Tetsuya Taga, Haruo Sugiyama, Tadamitsu Kishimoto, and Yoshiaki Sonoda

We assessed the biologic role of signaling through gp130, a signal-transducing receptor (R) component, in human hematopoiesis in vitro. Although peripheral blood-derived CD34$^+$ cells ubiquitously expressed gp130 and interleukin-3 receptor α (IL-3Rα), IL-6Rα was only detected on 80% of these CD34$^+$ cells. We sorted CD34$^+$IL-6R$^+$ or CD34$^+$IL-6R$^-$ cells and studied the effect on hematopoietic colony formation of signaling through gp130 activated by IL-6 or a combination of IL-6 and recombinant soluble human IL-6R (sIL-6R) in the presence or absence of stem cell factor (SCF) and/or IL-3. Signals activated by SCF, IL-6, or IL-6/sIL-6R complex alone did not induce significant colony formation. However, a combination of IL-3, SCF, and IL-6/sIL-6R complex dramatically induced many neutrophil (colony-forming unit-granulocyte [CFU-G]), erythroid burst (burst-forming unit-erythrocyte [BFU-E]), erythrocyte-containing mixed (CFU-Mix), and megakaryocyte (CFU-Meg) colony formations when CD34$^+$IL-6R$^-$ cells were used as the target. CFU-G colony formation induced by the three signals was more evident when CD34$^+$IL-6R$^+$ cells were used as the target. This distinct synergistic effect of the three different signals was confirmed by single-cell clone-sorting experiments. Moreover, colony formation (including CFU-G, BFU-E, CFU-Mix, and CFU-Meg) was observed even in the presence of neutralizing antibodies for granulocyte colony-stimulating factor, erythropoietin, and thrombopoietin (c-Mpl), whereas neutralizing antibodies for gp130, IL-6R, IL-3, and SCF partially or completely blocked the synergistic effect. The maturation of neutrophilic, erythroid, and megakaryocytic cells supported by the three signals in serum-free cultures was confirmed by immunostaining using anti-CD66b, antiglycophorin A, antihemoglobin α, and anti-CD41 monoclonal antibodies, respectively. In contrast, any two of the three signals were insufficient for effective blood cell production in the absence of maturation factors. These results suggest that simultaneous activation of the three signals through gp130, c-kit, and IL-3R can induce in vitro proliferation and differentiation of trilineage hematopoietic progenitors in the absence of terminally acting lineage-specific factors.

RECENTLY, OGAWA[1] proposed that the growth factors may be divided into three groups, including late-acting lineage-specific factors, intermediate-acting stage-specific factors, and early acting factors affecting the kinetics of cell-cycle dormant primitive stem cells (HSC). His colleagues used mapping studies of blast cell colony formation to show that interleukin-6 (IL-6), granulocyte colony-stimulating factor (G-CSF), IL-11, stem cell factor (SCF), leukemia inhibitory factor (LIF), and IL-12 acted synergistically with IL-3 in support of colony formation from murine HSC.[2-5] Interestingly, these early acting cytokines may be grouped together based on their functional similarities.[1] In addition, the combination of SCF, a ligand for type III receptor (R) tyrosine kinase (TK), and any of these early acting factors exerts a synergistic action on in vitro hematopoietic colony formation by human and murine cells.[1,6-8] Moreover, IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4, which are intermediate-acting lineage nonspecific factors,[1,9] showed a distinct synergistic effect on hematopoietic colony formation when combined with SCF or IL-11.[10-12] Based on these experimental data, he proposed that cytokines belonging to different groups, including early acting factors, stage-specific factors, and ligands for type III RTKs, may synergistically interact with each other to stimulate the proliferation and differentiation of primitive hematopoietic stem/progenitor cells.[1]

We previously reported that the combinations of SCF or a ligand for flt3 RTK (FL) with IL-3 or GM-CSF showed a distinct synergistic effect on multipotential progenitor cells.[11,13] In addition, we found that IL-4, which is also a stage-specific factor,[1] shows a distinct synergistic action with G-CSF in support of neutrophil colony formation[14] and with SCF in support of erythroid and multipotential colony formation.[15] In this context, we have studied interactions between the above-mentioned cytokines in human hematopoiesis in vitro. It is well documented that signals activated by early acting factors (including IL-6, IL-11, and LIF) are transmitted through a signal-transducing receptor component, gp130,[16-18] and that gp130 mRNA is ubiquitously expressed in a wide variety of cells, including hematopoietic stem/progenitor cells.[17] Therefore, we studied the role of gp130 signaling in the proliferation and differentiation of human hematopoietic progenitor cells using peripheral blood (PB)-derived highly purified CD34$^+$ cells. Recently, it was reported that signals through gp130 and c-kit promote erythropoietin (Epo)-independent erythrocyte production from umbilical cord blood (CB)- or bone marrow (BM)-derived

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Address reprint requests to Yoshiaki Sonoda, MD, The Department of Hygiene, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyoku, Kyoto 602, Japan.

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$CD34^+$ cells.[19] However, the combination of these two signals was insufficient to promote erythrocyte production in our culture system. Our results clearly showed that simultaneous activation of three signals through gp130, c-kit, and IL-3R dramatically induced in vitro proliferation and differentiation of trilineage hematopoietic progenitor cells independently of terminally acting lineage-specific factors, such as G-CSF, Epo, and thrombopoietin (TPO).

MATERIALS AND METHODS

Recombinant factors and neutralizing antibodies. Purified bacterially derived recombinant human IL-3, GM-CSF, G-CSF, and SCF, as well as purified Chinese hamster ovary (CHO) cell-derived recombinant human Epo, were generously supplied by Kirin Brewery Co Ltd (Tokyo, Japan). Purified recombinant human TPO was prepared by the TPO Production Group (Kirin Brewery Co Ltd, Maebashi, Japan) and was provided by Dr Hiroshi Miyazaki (Kirin Brewery). Purified bacterially derived recombinant human IL-6 was kindly provided by Ajinomoto Co Inc (Yokohama, Japan) and had a specific activity of 6×10^6 U/mg. Recombinant soluble human IL-6 receptor (sIL-6R) was prepared as reported previously.[20]

Three antihuman gp130 monoclonal antibodies (MoAbs), including GPX7, GPX22, and GPZ35, were prepared as described previously.[21] They recognized different epitopes on gp130 and were shown to inhibit the IL-6–induced association of gp130 and IL-6 receptors. Antihuman IL-6R MoAb (PM1) was prepared as described[22] and specifically inhibited binding between the IL-6R and IL-6. In preliminary titration experiments, a combination of three anti-gp130 MoAbs at 1 μg/mL and the anti–IL-6R MoAb at 5 μg/mL completely abrogated the colony formation induced by these signals in clonal cell culture (data not shown).

Rabbit polyclonal antihuman IL-3 and antihuman G-CSF antisera were kindly provided by Kirin Brewery. A 1:100 dilution of each antiserum neutralized 10 ng/mL and 20 ng/mL of the relevant factor in preliminary titration experiments (data not shown). Rabbit polyclonal antihuman TPO (anti-TPO), antihuman c-Mpl (anti–c-Mpl), and antihuman Epo (anti-Epo) Abs were also provided by Dr Takashi Kato (Kirin Brewery). The addition of greater than 10 μg/mL of anti-TPO Ab and greater than 2 μg/mL of anti–c-Mpl Ab completely abrogated the effect of 50 ng/mL TPO on megakaryocyte colony formation in preliminary titration experiments.[23] The addition of greater than 1 μg/mL of anti-Epo Ab completely blocked the effect of 2 U/mL Epo on erythroid burst formation (data not shown). A murine MoAb for human SCF was purchased from Genzyme Corp (Boston, MA) and 20 μg/mL of this Ab completely neutralized 20 ng/mL of SCF in our culture system (data not shown).

Cell preparation and staining with MoAbs. After informed consent was obtained, PB mononuclear (MN) cells were collected from 11 patients with testicular tumors by leukapheresis using a Fenwall CS-3000 (Fenwall Laboratories, Inc, Deerfield, IL), as reported elsewhere.[11,13,15] These cells contained an average of 5.9% ± 3.4% (n = 11) $CD34^+$ cells by flow cytometric analysis. The samples were washed twice with α-medium containing 5% fetal calf serum (FCS; Flow Laboratories, Inc, McLean, VA) and nonadherent (NA) cells were recovered after overnight culture on plastic dishes. The MNNA cell fraction was further enriched for null cells using nylon wool columns (Wako Pure Chemicals, Osaka, Japan) and rosette formation with neuraminidase-treated sheep erythrocytes, as reported elsewhere.[9] The resultant PB-derived null cells contained an average of 47.7% ± 23.5% (n = 11) $CD34^+$ cells. BM MNNA cells were used in part to study the effect of gp130 signaling on colony-forming unit-erythroid (CFU-E).

These null cells were washed twice with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS^-) containing 2% FCS (staining medium) and then were passed through a stainless steel mesh. Cells were pelleted before staining with the following MoAbs: fluorescein isothiocyanate (FITC)-conjugated HPCA-2 (mouse IgG_1 CD34 MoAb; Becton Dickinson Immunocytometry Systems, San Jose, CA); purified antihuman gp130 MoAb (AM64; mouse IgG_1)[21]; purified antihuman IL-6R MoAb (MT18; mouse IgG_1)[22]; purified anti–IL-3R MoAb (mouse IgG_1; Pharmingen, San Diego, CA); purified antihuman common β chain MoAb (mouse IgG_1; Pharmingen); purified antihuman glycophorin A (GPA; mouse IgG_1; Immunotech S.A., Marseille Cedex, France); purified antihuman c-kit MoAb (mouse IgM; Immunotech); and phycoerythrin (PE)-conjugated goat antimouse IgM (Immunotech). Purified antihuman gp130, IL-6R, IL-3R, GPA, and common β chain MoAbs were biotinylated as described elsewhere.[24] For staining of CD34 and c-kit antigens, incubation was performed with 20 μL of purified antihuman c-kit MoAb per 5×10^5 cells for 30 minutes at room temperature. After washing twice with the staining medium, the cells were incubated with 20 μL of PE-conjugated goat antimouse IgM per 5×10^5 cells for 30 minutes at room temperature. The cells were then washed twice with the staining medium and incubated with 10 μL of normal mouse serum on ice to block free binding sites of the PE-conjugated secondary Ab. After 15 minutes, staining was performed with 20 μL of HPCA-2 (FITC) per 10^6 cells on ice for 30 minutes. For detection of gp130, IL-6R, IL-3R, GPA, and common β chain, incubation was performed with 5 μg of the above-mentioned biotinylated MoAbs per 10^6 cells for 30 minutes on ice. After washing, cells were incubated with streptavidin-PE (Becton Dickinson) for 30 minutes on ice. Subsequently, all cells were washed twice with the staining medium and were kept on ice for cell sorting. Negative controls included unstained cells and cells only stained with the secondary MoAb or with an FITC-conjugated isotype control IgG or only with the streptavidin-PE.

Flow cytometry and cell sorting. All fluorescence-activated cell sorting (FACS) analyses were performed on a FACStar Plus or FACS Vantage (Becton Dickinson) equipped with an argon laser tuned at 488 nm, as reported elsewhere.[11] Sorting gates were established for intermediate-forward scatter (FSC) and low side scatter (SSC). A dual-parameter dot diagram displaying FITC (CD34) and PE (gp130, IL-6R, IL-3R, c-kit, and common β chain) fluorescence was then generated from the gated events. For part of the experiments, cultured cells were serially analyzed for the expression of GPA and gp130. Data acquisition was performed using FACStar Plus Research Software or CELLQuest (Becton Dickinson) and at least 20,000 events were analyzed for each sample. Sorting windows were established for $CD34^+$ cells expressing different levels of IL-6R (data not shown). We sorted $CD34^+$IL-$6R^+$ and $CD34^+$IL-$6R^-$ cells. The phenotypic purity of the sorted cells consistently exceeded 90% when checked using postsorting flow cytometric analysis. After sorting, the recovered cells were washed twice with α-medium and cultured as described later.

Clonal cell culture. Cultures were performed in 35-mm Lux suspension culture dishes (no. 171099; Nunc Inc, Naperville, IL), as reported elsewhere.[11,13,14] One milliliter of culture contained 200 sorted cells, a 1.2% concentration of 1,500 centipoise methylcellulose (Shinetsu Chemical, Tokyo, Japan), 30% FCS, 1% deionized fraction V bovine serum albumin (BSA; Sigma Chemical Co, St Louis, MO), 5×10^{-5} mol/L 2-mercaptoethanol (ME; Sigma), and one or more recombinant human CSFs. For megakaryocyte colony formation, 10% human platelet-poor plasma (PPP), which was prepared as reported elsewhere[23] and prescreened for its ability to support megakaryocyte colony formation, was used instead of FCS. The final concentrations of each CSF used were as follows: IL-3, 10 ng/mL; GM-CSF, 10 ng/mL; G-CSF, 20 ng/mL; Epo, 2 U/mL; SCF, 20 ng/mL; and TPO, 100 ng/mL. These concentrations supported

maximal total colony formation in preliminary titration experiments (data not shown).

Serum-free culture was performed as reported elsewhere.[9,11,15] Briefly, 200 sorted cells were plated in 35-mm Lux suspension culture dishes (Nunc) containing ASF 104 medium,[25] 1.2% methylcellulose, 1% globulin-free BSA (Sigma) that had been crystallized and deionized, 5×10^{-5} mol/L 2-ME, 300 μg/mL fully iron-saturated human transferrin (TF; 95% pure; Sigma), 10 μg/mL lecithin (Sigma), 6 μg/mL cholesterol (Sigma), and various growth factors. Dishes were incubated at 37°C in a fully humidified atmosphere flushed with a combination of 5% CO_2, 5% O_2, and 90% N_2. On days 12 through 14 of incubation, all colonies were counted under an inverted microscope. The typical morphologic features reported elsewhere[9,11,13-15,23] were used to identify megakaryocyte colonies (CFU-Meg), granulocyte colonies (CFU-G), macrophage colonies (CFU-M), granulocyte-macrophage colonies (CFU-GM), erythroid burst-forming units (BFU-E), eosinophil colonies (CFU-Eo), and erythrocyte-containing mixed (E-Mix) colonies (CFU-Mix). CFU-E–derived small erythroid colony was assayed on day 7 of incubation. In some experiments, we used megakaryocyte-containing mixed (M-Mix) colonies.

The average plating efficiencies of PB-derived $CD34^+IL\text{-}6R^+$ and $CD34^+IL\text{-}6R^-$ cells were 40% to 60% in the presence of a cocktail of CSFs, including SCF, IL-3, GM-CSF, G-CSF, and Epo (5 CSFs). However, the $CD34^+IL\text{-}6R^-$ cell population contained a large number of BFU-E, CFU-Mix, and CFU-Meg, whereas the $CD34^+IL\text{-}6R^+$ cell population contained largely CFU-G (Kimura et al, unpublished data). Our data are consistent with that of a recent report.[26]

Clone-sorting and single-cell culture. Clone-sorting was performed on a FACStar Plus using an Automated Cell Deposition Unit (ACDU), as reported previously.[15,23,27] Serum-containing liquid suspension cultures were performed in 96-well plates. Cultures consisted of single cells in wells containing α-medium, 1% BSA, 5×10^{-5} mol/L 2-ME, 30% FCS, and the designated factors. Incubation was performed for 14 days at 37°C in a fully humidified atmosphere flushed with a combination of 5% CO_2, 5% O_2, and 90% N_2. On days 5, 10, and 14, each well was scanned under an inverted microscope. When a positive well was identified, the number of cells per clone was directly counted in situ. Large clones containing greater than 500 cells were picked up on day 14 and the number of cells was counted using a counting chamber. Serum-containing methylcellulose cultures consisting of single cells were also performed as reported.[15,23,27]

Serum-free liquid suspension culture. PB-derived $CD34^+$ cells were cultured at 2×10^3/mL in 1 mL of ASF 104 medium[25] supplemented with 1% crystallized and deionized globulin-free BSA (Sigma), 5×10^{-5} mol/L 2-ME, 300 μg/mL fully iron-saturated human TF (Sigma), 10 μg/mL lecithin (Sigma), 6 μg/mL cholesterol (Sigma), and the optimum concentrations of the designated growth factors. Dishes were incubated at 37°C in a fully humidified atmosphere flushed with a combination of 5% CO_2, 5% O_2, and 90% N_2 for 14 days. Viable cells in each dish were counted on days 5, 7, 10, and 14 of incubation. Cytocentrifuged preparations were made for morphologic examination and confirmation of specific lineages, as described below.

Cytochemistry and immunostaining. Cells were harvested from representative colonies or suspension cultures. Cytospin preparations were first stained with May-Gruenwald-Giemsa (M-G) staining for morphologic analysis. Confirmation of the neutrophilic, erythroid, and megakaryocytic nature of the cells was obtained by immunostaining with the alkaline phosphatase antialkaline phosphatase (APAAP) method using the following MoAbs: anti-CD66b (mouse IgG_1; Immunotech), anti-GPA (mouse IgG_1, Immunotech), anti-hemoglobin (Hb) α (mouse IgG_1; Cosmo Bio, Tokyo, Japan), and anti-CD41 (mouse IgG_1; Immunotech). Staining was performed using a DAKO APAAP KIT (Dakopatts, Osaka, Japan) according to the instructions of the manufacturer. The final preparations were counterstained with Mayer's hematoxylin (Wako Pure Chemicals).

Determining the size of pure megakaryocyte colonies and the diameter of megakaryocytes. Colony size was estimated through counting the number of cells in pure megakaryocyte colonies by direct microscopic visualization. Pure megakaryocyte colonies were then picked up with a fine Pasteur pipette and spread on glass slides using cytocentrifugation. After immunostaining with anti-CD41 MoAb (APAAP method), the diameters of individual positive cells were measured using a microscope equipped with an ocular micrometer, and the mean of two perpendicular diameters of each cell was calculated.

Assessment of megakaryocyte ploidy. Cytospin preparations of pooled pure megakaryocyte colonies were made as described elsewhere.[23] Pooled cells were confirmed to be positive for CD41 before use. Slides were fixed in cold 70% ethanol at 4°C. After treatment with RNase A (0.1% in PBS^-; Sigma), the slides were stained with 0.0025% propidium iodide (PI; Sigma) for 15 minutes at 4°C and mounted with 0.0025% PI solution. The relative nuclear DNA content of cultured megakaryocytes was then individually determined using epifluorecent microfluorometry.

Statistical analysis. The significance of differences in mean values was determined using the two-tailed Student's *t*-test, the Mann-Whitney rank-sum test, or the χ^2 test.

RESULTS

Characterization of PB-derived $CD34^+$ cells. To characterize PB-derived $CD34^+$ cells, we investigated the expression of various cell surface antigens/receptors by these cells. As reported elsewhere,[27] the majority of PB-derived $CD34^+$ cells expressed HLA-DR and CD38 antigens. In contrast, approximately 60% and 20% of the $CD34^+$ cells expressed CD33 and c-kit antigens, respectively. Interestingly, PB-derived $CD34^+$ cells ubiquitously expressed gp130 and IL-3R. In addition, most of the cells showed weak common β chain expression. On the other hand, 80% of the $CD34^+$ cells expressed IL-6R (data not shown). We used $CD34^+IL\text{-}6R^+$ and $CD34^+IL\text{-}6R^-$ cells in the following experiments.

Effect of signals activated by IL-6/sIL-6R complex on colony formation by PB-derived $CD34^+$ $IL\text{-}6R^-$ or $CD34^+IL\text{-}6R^+$ cells in the presence or absence of SCF and/or IL-3. First, we investigated effect of signals mediated through gp130 and activated by IL-6/sIL-6R complex on colony formation using 200 PB-derived $CD34^+IL\text{-}6R^-$ cells as the target in serum-containing as well as serum-free culture. The pattern of colony formation observed in serum-free culture in the presence of each signal was comparable with that in serum-containing culture. Therefore, representative data from six independent experiments in serum-free culture are presented in Table 1. The signals activated by IL-6/sIL-6R and SCF alone did not induce significant colony formation. However, IL-3 alone supported significant eosinophil colony formation, as reported elsewhere.[9] When IL-3 and IL-6/sIL-6R were combined, only a few erythroid burst formations were observed in addition to eosinophil colonies. Combinations of SCF and IL-3, and of SCF and IL-6/sIL-6R induced a small number of neutrophil colonies. Interestingly, a combination of SCF, IL-3, and IL-6/sIL-6R dramatically induced variety of colonies derived from CFU-G, CFU-GM, BFU-E, and CFU-Mix. As described above, CFU-G was enriched

Table 1. Colony Formation by PB-Derived CD34⁺IL-6R⁻ Cells in Serum-Free Culture

Factors	Colony Types							% of Control
	G	M	GM	B	Eo	E-Mix	Total	
None	0	0	0	0	0	0	0	0.0
SCF	1 ± 1	0	0	0	0	0	1 ± 1	1.0
IL-3	0	0	2 ± 1	0	8 ± 1	0	9 ± 0	8.7
IL-6 + sIL-6R	0	0	0	0	0	0	0	0.0
SCF + IL-3	4 ± 2 (NS)	0	5 ± 2	0	5 ± 1	0	14 ± 3	13.6
SCF + IL-6 + sIL-6R	2 ± 1 (NS)	0	2 ± 0	1 ± 0 (NS)	0	2 ± 1 (NS)	6 ± 0	5.8
IL-3 + IL-6 + sIL-6R	0	0	4 ± 1	2 ± 0 (NS)	6 ± 1	1 ± 0 (NS)	12 ± 0	11.7
SCF + IL-3 + IL-6 + sIL-6R	8 ± 1*	0	5 ± 1	52 ± 3†	5 ± 2	19 ± 1†	88 ± 3	85.4
CSFs	11 ± 1	1 ± 1	18 ± 7	55 ± 6	2 ± 1	18 ± 2	130 ± 9	100.0

Data represent the mean ± SD of duplicate cultures containing 200 CD34⁺IL-6R⁻ cells/dish. CSFs contained SCF, IL-3, GM-CSF, G-CSF, and Epo.

Abbreviations: G, granulocyte; M, macrophage; GM, granulocyte-macrophage; B, erythroid burst; Eo, eosinophil; E-Mix, erythrocyte-containing mixed colony; NS, not significant.

* $P < .02$.

† $P < .01$.

in the CD34⁺IL-6R⁺ cell fraction. Therefore, the synergistic action of the combination of SCF, IL-3, and IL-6 on neutrophil colony formation was more evident when this population of progenitors was used as the target (see Table 3A). In the separate experiment, the effect of gp130 signaling on CFU-E was investigated. The combination of IL-6/sIL-6R supported a small but significant number of CFU-E. The combinations of IL-3 + IL-6/sIL-6R, SCF + IL-6/sIL-6R, and three signals significantly enhanced CFU-E colony formation, implying the presence of gp130, c-kit, and IL-3R on CFU-E progenitors (data not shown).

The neutrophilic and erythroid nature of the cells was confirmed by immunostaining for CD66b, GPA, and Hbα. The number of colonies supported by the three signals was approximately 85% of that supported by a combination of SCF, IL-3, GM-CSF, G-CSF, and Epo (5 CSFs). In a series of experiments, 100 U/mL of IL-6, 1 μg/mL of sIL-6R, and 20 ng/mL of SCF were added to the cultures based on the data obtained by preliminary titration experiments. These concentrations supported maximal colony formation (data not shown).

Our data indicate that the combination of SCF + IL-3 + IL-6/sIL-6R can induce not only erythroid burst and erythrocyte-containing mixed colonies, but also neutrophil colony in the absence of Epo or G-CSF. Moreover, single-cell clone-sorting experiments performed in methylcellulose culture provided strong evidence that the dramatic synergistic action of the three signals on hematopoietic colony formation is independent of the presence of accessory cells (data not shown).

Effect of signals activated by IL-6/sIL-6R complex on megakaryocyte colony formation by PB-derived CD34⁺ IL-6R⁻ cells in the presence or absence of SCF and/or IL-3. Next, we investigated the effects of combinations of two or three signals activated by SCF, IL-3, and IL-6/sIL-6R complex on megakaryocyte colony formation in plasma-containing as well as serum-free culture. Representative data of three independent experiments performed in serum-free culture are shown in Table 2. The combination of any two of the three signals activated by SCF, IL-3, and IL-6/sIL-6R supported a comparable number of pure and mixed megakaryocyte colonies. The most striking colony formation was obtained in the presence of SCF + IL-3 + IL-6/sIL-6R, with almost all of them being megakaryocyte-containing mixed colonies. The megakaryocytic nature of the cells was confirmed by M-G staining and immunostaining with anti-CD41 MoAb. In contrast, TPO alone only supported pure megakaryocyte colonies. These results indicate that the signals activated by SCF, IL-3, and IL-6/sIL-6R complex supported megakaryocyte colony formation independently of the presence of TPO.

Effect of neutralizing antibodies or antisera on colony formation by PB-derived CD34⁺IL-6R⁺ or CD34⁺IL-6R⁻ cells. To confirm that the signals activated by SCF, IL-3, and IL-6/sIL-6R complex actually supported CFU-G, BFU-E, and CFU-Meg independently of G-CSF, Epo, and TPO, we investigated the effect on colony formation of neutralizing Abs (antiserum) for G-CSF, Epo, and TPO (c-Mpl). The synergistic action of the three signals on CFU-G and BFU-E colonies in the presence of neutralizing Abs or antisera for SCF, IL-3, IL-6R, and gp130 was also studied. CFU-G was highly enriched in the CD34⁺IL-6R⁺ cell population,

Table 2. Pure and Mixed Megakaryocyte Colony Formation by PB-Derived CD34⁺IL-6R⁻ Cells in Serum-Free Culture

Factors	Colony Types			
	Meg	M-Mix	Others	Total
TPO	140	0	0	140
SCF	0	0	5	5
IL-3	35	24	131	190
SCF + IL-3	38	32	227	297
SCF + IL-6 + sIL-6R	47	24	76	147
IL-3 + IL-6 + sIL-6R	20	57	332	409
SCF + IL-3 + IL-6 + sIL-6R	7	135	607	749

Data represent the total number of colonies derived from 10 dishes each containing 200 CD34⁺IL-6R⁻ cells.

Abbreviations: Meg, pure megakaryocyte colony; M-Mix, megakaryocyte-containing mixed colony.

Table 3. Effects of Neutralizing Antibodies or Antisera on Colony Formation by PB-Derived CD34⁺IL-6R⁺ CD34⁺IL-6R⁻ Cells

Factors	Antibodies or Antisera	Colony Types G	M	GM	B	Eo	E-Mix	Total
A) CD34⁺IL-6R⁻ cells								
SCF + IL-3	—	7 ± 1	8 ± 1	3 ± 2	0	13 ± 2	0	32 ± 1
SCF + IL-6	—	7 ± 1	3 ± 2	0	0	0	0	9 ± 1
IL-3 + IL-6	—	0	11 ± 3	3 ± 2	0	18 ± 1	0	31 ± 2
SCF + IL-3 + IL-6	—	18 ± 2	8 ± 2	5 ± 3	7 ± 2	11 ± 1	2 ± 1	51 ± 3
	Control sera*	19 ± 2 (NS)	7 ± 4	6 ± 1	7 ± 2	11 ± 2	1 ± 0	51 ± 1
	Anti-G-CSF serum†	21 ± 2 (NS)	6 ± 2	4 ± 1	7 ± 2	12 ± 1	1 ± 1	51 ± 2
	Anti-SCF Ab‡	0#	11 ± 2	3 ± 1	0	18 ± 2	0	32 ± 3
	Anti-IL-3 serum†	5 ± 1**	4 ± 1	0	0	0	0	9 ± 0
	Anti-IL-6R Ab§	6 ± 2**	10 ± 1	3 ± 1	0	15 ± 2	0	34 ± 4
	Anti-gp130 Abs‖	8 ± 1**	8 ± 4	4 ± 1	0	15 ± 3	0	34 ± 5
	Combination of Abs¶	0#	0	0	0	0	0	0
	CSFs	40 ± 2	12 ± 1	3 ± 1	41 ± 3	6 ± 2	3 ± 1	105 ± 3
(B) CD34⁺IL-6R⁻ cells								
SCF + IL-3	—	4 ± 1	5 ± 1	3 ± 2	0	12 ± 2	0	24 ± 1
SCF + IL-6 + sIL-6R	—	2 ± 1	4 ± 2	0	3 ± 1	0	2 ± 1	18 ± 2
IL-3 + IL-6 + sIL-6R	—	0	5 ± 2	3 ± 1	14 ± 1	3 ± 1	7 ± 1	32 ± 2
SCF + IL-3 + IL-6 + sIL-6R	—	5 ± 1	4 ± 1	3 ± 2	51 ± 3	4 ± 1	23 ± 1	90 ± 4
	Control sera	5 ± 1	3 ± 2	2 ± 1	52 ± 2 (NS)	4 ± 2	24 ± 1	87 ± 3
	Anti-Epo Ab§	4 ± 2	3 ± 2	4 ± 1	50 ± 2 (NS)	3 ± 2	25 ± 1	89 ± 4
	Anti-SCF Ab	0	5 ± 1	3 ± 1	16 ± 1**	4 ± 2	7 ± 2	33 ± 1
	Anti-IL-3 serum	2 ± 1	3 ± 1	0	1 ± 1#	0	1 ± 1	7 ± 3
	Anti-IL-6R Ab	4 ± 2	6 ± 2	3 ± 1	0#	12 ± 2	0	24 ± 4
	Anti-gp130 Ab	4 ± 2	5 ± 1	5 ± 2	0#	13 ± 1	0	26 ± 3
	Combination of Abs	0	0	0	0#	0	0	0
	CSFs	14 ± 2	6 ± 2	5 ± 2	60 ± 4	4 ± 2	18 ± 2	106 ± 1

Data represent the mean ± SD of triplicate cultures containing 200 cells/dish with the specified factors and 30% FCS. Abbreviations are defined in the legend for Table 1.

* Normal mouse serum + normal rabbit serum.

† (1:100) dilution.

‡ 20 μg/mL.

§ 5 μg/mL.

‖ Combination of three anti-gp130 Abs (GPX-7, GPX-22, and GPZ-35) at 1 μg/mL each.

¶ Anti-SCF Ab + anti-IL-3 serum + anti-IL-6R Ab + anti-gp130 Abs.

# $P < .001$.

** $P < .01$.

so the effect of neutralizing Abs on neutrophil colony formation was studied using this population of progenitors, whereas the CD34⁺IL-6R⁻ cell population was used to study effects of neutralizing Abs on BFU-E and CFU-Meg (M-Mix).

As shown in Table 3A, anti-IL-3 serum, anti-IL-6R Ab, and anti-gp130 Abs significantly reduced the number of CFU-G supported by the three signals. In contrast, anti-SCF Ab and a combination of all of the Abs completely abrogated neutrophil colony formation, suggesting that signaling through c-kit plays a pivotal role in neutrophil production. However, the addition of anti-G-CSF serum did not affect colony formation, indicating that the three signals could support CFU-G independently of the presence of G-CSF. The effect of these neutralizing Abs on erythroid burst formation was then investigated using CD34⁺IL-6R⁻ cells (Table 3B). The addition of anti-IL-3 serum, anti-IL-6R Ab, anti-gp130 Abs, and a combination of all of the Abs completely abrogated erythroid burst formation. The number of BFU-E supported by the three signals did not change even in the presence of an anti-Epo Ab, indicating that the observed erythroid burst formation was independent of Epo.

Finally, we studied the effect of these neutralizing Abs on megakaryocyte colony formation. Anti-SCF Ab, anti-IL-6R Ab, and anti-gp130 Abs significantly reduced the number of M-Mix colonies supported by the three signals. In contrast, anti-IL-3 serum and a combination of all of the Abs completely abrogated colony formation. However, the addition of anti-TPO Ab or anti-c-Mpl Ab did not affect megakaryocyte colony formation, indicating that the three signals could support M-Mix colonies independently of the presence of TPO (data not shown).

Alternatively, the addition of anti-gp130 MoAbs to the cultures containing SCF + IL-3 + G-CSF or EPO, or TPO

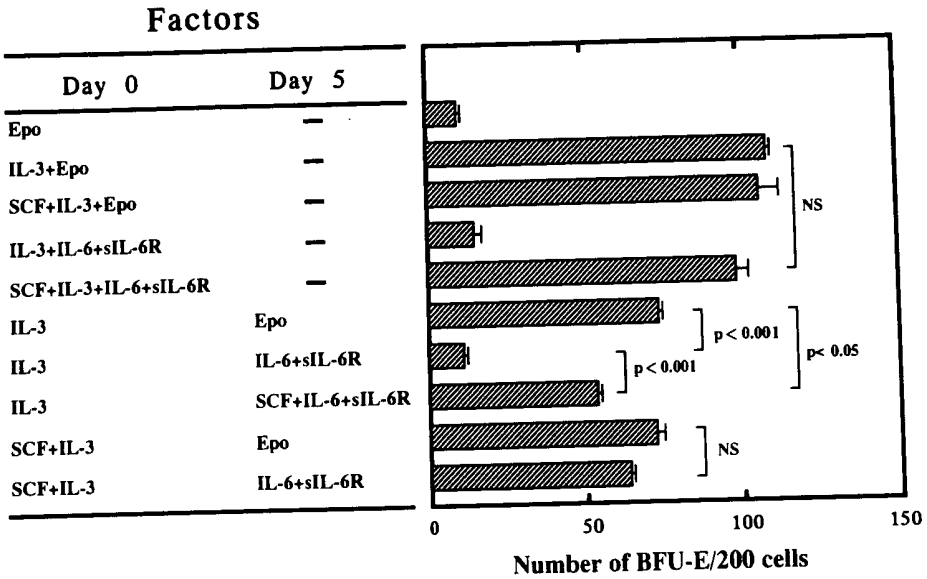

Fig 1. Effects of delayed addition of Epo, IL-6/sIL-6R, or SCF + IL-6/sIL-6R on erythroid burst formation by 200 PB-derived $CD34^+IL\text{-}6R^-$ cells supported by IL-3 or SCF + IL-3 in serum-containing cultures. (▨) The mean ± SD of the number of BFU-E is shown. Statistical analysis was performed using the two-tailed Student's *t*-test. NS, not significant.

alone, did not affect colony formation of these terminally acting factors (data not shown).

These results clearly showed that the observed synergistic action was initiated through interaction of the IL-6/sIL-6R complex with membrane-anchored gp130 and further confirmed that the three signals activated by SCF, IL-3, and IL-6/sIL-6R complex synergistically support committed progenitors, including CFU-G, BFU-E, CFU-Mix, and CFU-Meg (M-Mix) independently of the relevant lineage-specific factors such as G-CSF, Epo, and TPO.

Effect of delayed addition of Epo or IL-6/sIL-6R or SCF + IL-6/sIL-6R on erythroid burst formation by PB-derived $CD34^+IL\text{-}6R^-$ cells supported by IL-3 or SCF + IL-3 in serum-containing culture. The hematopoietic action of signaling through gp130 was further investigated by the delayed addition of Epo or IL-6/sIL-6R or SCF + IL-6/sIL-6R on day 5 of cultures containing IL-3 or SCF + IL-3 from day 0. Representative data for three independent experiments are shown in Fig 1. The number of BFU-E supported by SCF, IL-3, and IL-6/sIL-6R from day 0 was almost comparable with that supported by IL-3 (the most potent burst-promoting activity)[11] plus Epo. When Epo was added on day 5 to cultures containing IL-3 from day 0, approximately 70% of the BFU-E survived relative to those supported by day 0 IL-3 + EPO. In contrast, most of the BFU-E failed to recover when IL-6/sIL-6R was added on day 5 instead of Epo. When SCF + IL-6/sIL-6R was added on day 5 to cultures containing IL-3 from day 0, a significant number of BFU-E recovered, although the number of BFU-E was still significantly smaller than that supported by day 0 IL-3 followed by day 5 Epo. Interestingly, when IL-6/sIL-6R was added on day 5 to cultures containing SCF + IL-3 from day 0, a significant number of BFU-E recovered, indicating that signaling through gp130 activated by IL-6/sIL-6R induced maturation of BFU-E in the presence of SCF plus IL-3, but not in the presence of IL-3 alone. In other words, these results suggest that both IL-3 and SCF are key cytokines for the survival and/or proliferation of BFU-E and that signaling through gp130 is mainly important for terminal maturation.

Pattern of proliferation of single $CD34^+IL\text{-}6R^-$ cells in liquid suspension culture. Next, the hematopoietic action of the signals activated by SCF, IL-3, and IL-6/sIL-6R complex was further investigated using single-cell suspension cultures. Single PB-derived $CD34^+IL\text{-}6R^-$ cells were deposited in wells and the pattern of proliferation was serially observed on days 5, 10, and 14 using an inverted microscope. Wells containing greater than 8 cells were scored as positive clones and are shown in Fig 2. SCF and IL-6/sIL-6R complex alone did not induce significant proliferation of single cells. IL-3 alone did induce proliferation of single $CD34^+IL\text{-}6R^-$ cells, but half of the clones disappeared on day 10. In the presence of SCF + IL-3 or IL-3 + IL-6/sIL-6R, the proliferation of single $CD34^+IL\text{-}6R^-$ cells was enhanced compared with that in the presence of IL-3 alone. Subsequently, the number of clones proliferating markedly decreased on day 14 in cultures containing SCF + IL-3, suggesting that the clones died during culture. In cultures containing IL-3 + IL-6/sIL-6R, 80% of the clones still survived on day 14, suggesting that the cells had differentiated along with the maturation process. In contrast, the number of clones was smaller in the cultures containing SCF + IL-6/sIL-6R, but the positive wells were maintained on day 14 of culture. The most striking proliferation of single $CD34^+IL\text{-}6R^-$ cells was observed in cultures containing SCF + IL-3 + IL-6/sIL-6R. Half of the clones contained greater than 500 cells on day 14 of culture, and the number of positive wells did not change during the observation period. Huge clones of greater than 1,000 cells were only observed in the presence of the three signals. These results again showed a distinct synergistic action of the three signals activated by SCF, IL-3, and IL-6/sIL-6R complex on the proliferation of hematopoietic progenitors at the single progenitor cell level.

Effects of the three signals activated by IL-3, SCF, and IL-6/sIL-6R complex on proliferation and differentiation of megakaryocyte progenitor cells in serum-free culture. We studied the effect of the three signals activated by IL-3,

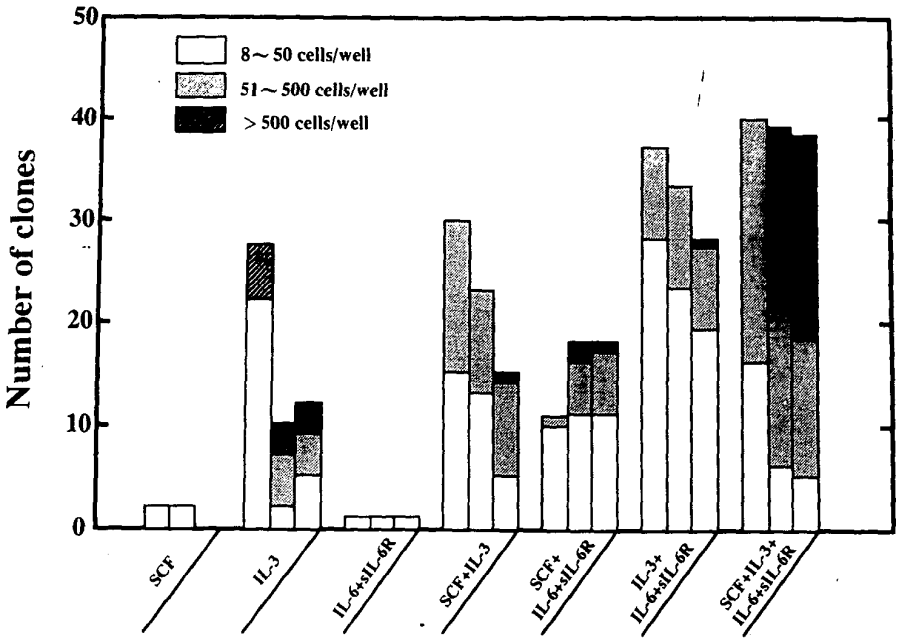

Fig 2. Proliferation of single PB-derived CD34⁺IL-6R⁻ cells deposited in the wells of a 96-well flat-bottomed microtiter plate in the presence of the designated factors was serially analyzed on day 5 (left bar), day 10 (center bar), and day 14 (right bar) of culture. Each well was scanned under an inverted microscope. The number of cells per clone was directly counted in situ and wells containing greater than 8 cells were scored as positive clones. Large clones containing greater than 500 cells were picked up on day 14 and the number of cells was counted using a counting chamber. Shown are the clones of (□) 8 to 50, (▨) 51 to 500, and (▩) greater than 500 cells.

SCF, and IL-6/sIL-6R complex singly or in combination on megakaryocyte colony formation in serum-free cultures. The mean number of megakaryocytes in individual colonies supported by IL-3 was 54.4 ± 12.2 cells. A combination of SCF + IL-3 or SCF + IL-3 + IL-6/sIL-6R significantly increased the size of the colonies to 70.7 ± 16.0 ($P < .05$) and 91.6 ± 15.6 ($P < .01$) cells, respectively.

On the other hand, the mean diameter of megakaryocytes supported by IL-3 alone was 24.6 ± 8.0 μm. SCF did not enlarge megakaryocytes, but the signal through gp130 activated by IL-6/sIL-6R complex significantly increased the mean diameter of megakaryocytes in the presence of IL-3 or SCF. In the presence of the three signals, the mean diameter significantly increased to 48.8 ± 20.3 μm ($P < .02$). However, the mean diameter was still significantly less than that (60.6 ± 19.6 μm) supported by TPO alone ($P < .001$).

Next, we examined the nuclear DNA content of megakaryocytes in pure megakaryocyte colonies to study the effect of the three signals on megakaryocytic maturation (Table 4). Approximately 70% of the megakaryocytes supported by IL-3 or SCF + IL-3 were 2 N in ploidy and there was no significant difference of the mean DNA content. In contrast, the mean ploidy of megakaryocytes was significantly increased in the presence of IL-6/sIL-6R complex. In the presence of three signals, the mean DNA content was 6.62 ± 7.35 N and higher ploidy classes (16 N and 32 N) showed a significant increase ($P < .01$). However, the mean DNA content was still significantly less than that supported by TPO alone ($P < .01$).

Based on these data, it appears that SCF promoted the proliferation of CFU-Meg initially supported by IL-3, whereas the predominant effect of the signal through gp130 activated by IL-6/sIL-6R complex was induction of megakaryocyte maturation.

Serial analysis of the expression of GPA on the surface of cultured cells in the presence of the three signals or SCF + IL-3+ Epo in serum-free culture. In this experiment, 2 × 10^3 CD34⁺IL-6R⁻ cells were cultured per dish in the presence of SCF + IL-3 + IL-6/sIL-6R or SCF + IL-3 + Epo. The expression of GPA on the surface of cultured cells was serially analyzed on days 7, 10, and 14 of culture by flow cytometry. The expression of GPA gradually increased in both culture conditions. However, the peak fluorescence intensity on day 14 was much higher in the culture supported by SCF + IL-3+ Epo than that supported by the three signals (data not shown). Moreover, cytologic analysis of cytospin preparations of cultured cells stained with May-Gruenwald-Giemsa showed that the percentage of erythroid cells (erythroblasts + erythrocytes) was 84.5% in the presence of SCF + IL-3 + Epo, whereas that was only 33.5% in the presence of the three signals. The absolute number of erythrocytes produced in the culture containing SCF + IL-3 + Epo was approximately 5 times more than that produced in the culture supported by the three signals (3.42 × 10^4 *v* 1.61 × 10^5 cells). These results clearly indicated that Epo induces the maturation of erythroid cells more efficaciously than does the signal through gp130.

Serial analysis of the maturation of progenitors supported by IL-3, SCF, and IL-6/sIL-6R singly and/or in combination in serum-free liquid suspension culture. Finally, we examined the growth pattern of CD34⁺IL-6R⁻ and CD34⁺IL-6R⁺ cells in serum-free liquid suspension culture, and serially analyzed the number of CD41⁺, GPA⁺, Hbα⁺, and CD66b⁺ cells using the APAAP method on days 5, 7, 10, and 14 of culture. In these experiments, 2 × 10^3 CD34⁺IL-6R⁻ or CD34⁺IL-6R⁺ cells were cultured per dish in the presence of the designated factors. Representative data from two experiments are shown in Figs 3 and 4. The pattern of proliferation of CD34⁺IL-6R⁻ cells (Fig 3) was almost identical with that seen in single-cell culture (Fig 2). IL-3 supported the

Table 4. Ploidy Distribution of Megakaryocytes Supported by Specified Factors in Serum-Free Culture

Factors	No. of Megakaryocytes Analyzed	Ploidy Class (%)*					Mean ± SD
		2N	4N	8N	16N	32N	
IL-3	150	72.7	19.3	7.3	0.7		2.92 ± 1.97
SCF + IL-3 (NS)	150	66.7	23.3	10.0			3.07 ± 1.84
SCF + IL-6 + sIL-6R†	150	54.0	26.0	20.0			3.72 ± 2.30
IL-3 + IL-6 + sIL-6R‡	200	48.5	29.0	12.5	10.0		4.63 ± 4.22
SCF + IL-3 + IL-6 + sIL-6R§	200	44.0	18.5	23.5	8.5	5.5	6.62 ± 7.35
TPO§	150	19.3	22.0	28.7	24.7	5.3	9.21 ± 7.45

One hundred fifty or 200 megakaryocytes were analyzed for relative nuclear DNA content. Statistical significance was calculated by the χ^2 test.

Abbreviation: NS, not significant.

* Percentage of megakaryocytes in each ploidy class. Ploidy distribution was compared between IL-3 and the specified factors. The mean nuclear DNA content supported by the three signals was significantly less than that supported by TPO ($P < .01$). Granulocytes obtained from pure neutrophilic colonies formed in the cultures were used as the diploid standards.

† $P < .05$.

‡ $P < .02$.

§ $P < .01$.

proliferation of $CD34^+IL\text{-}6R^-$ cells and SCF significantly promoted their growth. In the presence of the three signals, proliferation was most striking and the number of cells reached approximately 9×10^5/dish on day 14 (Fig 3A). In the presence of SCF + IL-3 or IL-3 + IL-6/sIL-6R, approximately 1.5×10^4 $CD41^+$ cells were observed on day 7. However, they markedly decreased in the presence of SCF + IL-3 on day 14. In contrast, $CD41^+$ cells could be maintained in the presence of IL-3 + IL-6/sIL-6R. Moreover, the number of $CD41^+$ cells supported by the three signals was twice that supported by IL-3 + IL-6/sIL-6R (Fig 3B).

On the other hand, SCF + IL-3 could not induce GPA^+

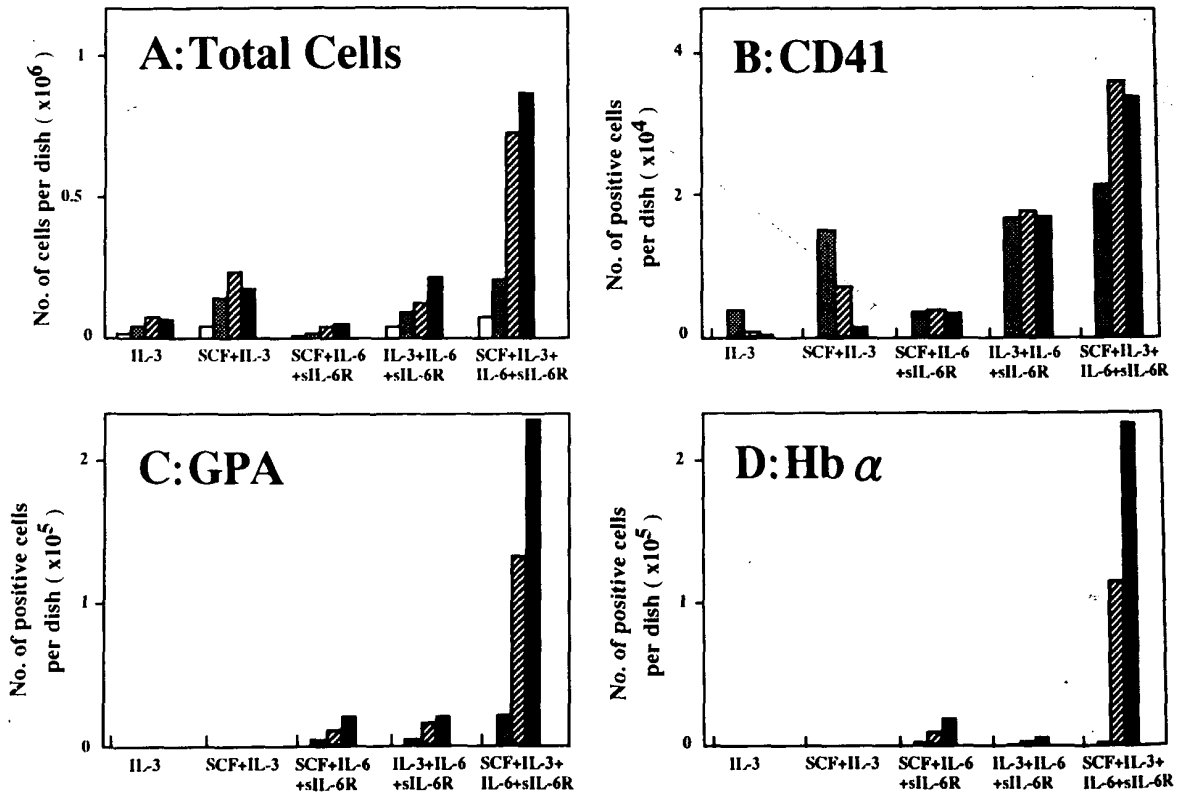

Fig 3. Number of viable cells (A) and number of cells positive for immunostaining with CD41 (B), GPA (C), or Hbα (D) on days 5, 7, 10, and 14 of culture in the presence of the designated factors. Cultures were initiated with 2×10^3 PB-derived $CD34^+IL\text{-}6R^-$ cells per dish. Shown are the numbers of cells on days (□) 5, (■) 7, (▨) 10, and (■) 14.

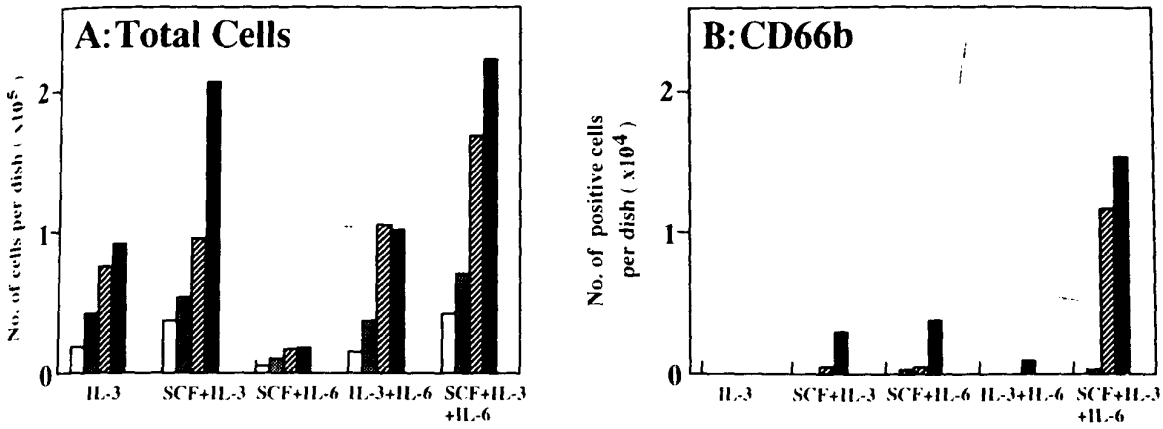

Fig 4. **Number of viable cells (A) and number of cells positive for immunostaining with CD66b (B) on days 5, 7, 10, and 14 of culture in the presence of the designated factors. Cultures were initiated with 2 × 10^3 PB-derived $CD34^+IL\text{-}6R^+$ cells per dish. Shown are the numbers of cells on days (□) 5, (▩) 7, (▨) 10, and (■) 14.**

and $Hb\alpha^+$ cells under the same culture conditions (Fig 3C and D). In the presence of SCF + IL-6/sIL-6R or IL-3 + IL-6/sIL-6R, a few GPA^+ and $Hb\alpha^+$ cells could be detected. Interestingly, in the presence of the three signals, greater than 2 × 10^5 cells were positive for GPA and Hbα immunostaining on day 14. Approximately 98.5% of the $CD34^+IL\text{-}6R^-$ cells were blastoid in morphology at the initiation of culture, but 24.0% and 5.5% of the cells were respectively erythroblasts and mature erythrocytes on day 14. This findings was highly consistent with the immunostaining data. These results clearly indicated that signaling through gp130 significantly induced maturation of megakaryocytic as well as erythroid progenitor cells in the presence of IL-3 or SCF + IL-3.

When $CD34^+IL\text{-}6R^+$ cells were cultured under the same conditions, the number of cells reached a peak level (2 × 10^5/dish) on day 14 in both the presence of SCF + IL-3 and SCF + IL-3 + IL-6 (Fig 4A). However, only in the presence of the three signals did 7% of the cells become $CD66b^+$ on day 14 (Fig 4B), which was consistent with morphologic examination by M-G staining. These results again showed that signaling through gp130 may play an important role on the maturation of neutrophilic progenitor cells in the presence of SCF and IL-3. Our findings further suggested simultaneous activation of the three signals through gp130, c-kit, and IL-3R induced not only proliferation, but also maturation of neutrophilic, erythroid, and megakaryocytic progenitor cells in the absence of terminally acting factors, including G-CSF, Epo, and TPO.

DISCUSSION

An important finding of this study is that a combination of three signals through gp130, c-kit, and IL-3R exerted a dramatic synergistic action on hematopoietic colony formation. In the presence of the three signals, committed and multipotential progenitors (including CFU-G, BFU-E, CFU-Meg, and CFU-Mix) could proliferate and differentiate to form colonies in serum-free culture in the absence of terminally acting lineage-specific factors, such as G-CSF, Epo, and TPO. Single-cell clone-sorting experiments in liquid suspension culture clearly showed that all three signals were essential for a dramatic synergistic effect on the proliferation and differentiation of hematopoietic progenitor cells. Moreover, data from single-cell suspension cultures suggested that IL-3 support was important for survival and initial proliferation, whereas SCF was important for enhancement of the proliferation of progenitors. The signal through gp130 activated by IL-6/sIL-6R complex may mainly contribute to the maturation of progenitors in the presence of SCF and IL-3, as suggested by the data from delayed addition experiments and serum-free suspension cultures. In addition, approximately 95% of mature hematopoietic cells, which are derived from the day-14 culture supported by the three signals and consist of 32.5% myeloid cells, 21.0% monocytes, 9.0% eosinophils and basophils, 33.5% erythroid cells, and 2% megakaryocytes, expressed gp130 by flow cytometric analysis (Kimura et al, unpublished data). These findings further support that signals through gp130 function as the maturation stimuli.

Our interpretation of the role of SCF is consistent with an earlier report that it enhances the proliferation but not maturation of murine megakaryocyte progenitors.[28] A maturation-promoting effect of IL-6 on megakaryocytes has already been reported.[29] Moreover, the IL-6 family of cytokines, including IL-6, IL-11, LIF, and oncostatin M, uses gp130 as a signal transducer and functions as a megakaryocyte-potentiating activity.[18] Subsequently, Debili et al[30] clearly indicated that a combination of SCF, IL-6, and IL-3 promoted the maturation of CFU-Meg and led to the synthesis of demarcation membranes and platelet shedding, further supporting our results. Moreover, Papayannopoulou et al[31] reported that SCF exerted synergy with IL-3 in supporting the survival and/or amplification of early and late erythroid progenitors in the absence of Epo. These erythroid progenitors could differentiate to the stage of globin-producing cells, but were unable to complete the maturation process, includ-

ing GPA expression. These results suggest that two signals through c-kit and IL-3R are important for BFU-E development, but are insufficient for the terminal maturation of erythroid progenitors, and that Epo is not critical for the generation of globin$^+$ cells in the presence of IL-3 + SCF. Our data are consistent with their conclusions and suggest that signaling through gp130 can partially substitute for Epo and induce further maturation of erythroid progenitors. The effects of SCF and SCF + IL-3 on myeloid colony formation have been reported previously,[12,32] but it was still uncertain whether signaling through gp130 promotes the maturation of neutrophilic progenitors. Our immunostaining study using anti-CD66b MoAb, which detects mature neutrophils, clearly showed that the three signals greatly enhanced the maturation of neutrophilic progenitors in serum-free cultures.

The present study provides an evidence that signals through gp130 induced the maturation of trilineage hematopoietic progenitor cells in the presence of SCF and IL-3. However, the mean DNA content of the megakaryocytes supported by the three signals was still significantly less than that supported by TPO alone, suggesting that the signaling through gp130 is not as effective as TPO. Moreover, the expression of GPA on the surface of mature erythroid cells obtained in the culture supported by the three signals was apparently less than that in the culture supported by SCF + IL-3 + Epo. These results indicate that signals through gp130 cannot replace the function of physiologic maturation factors completely.

Very recently, Sui et al[33] reported that the IL-6/sIL-6R complex shows distinct synergy with SCF in promoting the proliferation of CB-derived CD34$^+$ cells. It was also reported that signaling through gp130 and c-kit dramatically promoted erythropoiesis from human CB- and BM-derived CD34$^+$ cells.[19] However, we could not induce such a potent hematopoietic action by these two signals in our cultures. Our data clearly indicated that signaling through IL-3R has a pivotal role and that a combination of the three signals through gp130, c-kit, and IL-3R exerts a dramatic hematopoietic action in vitro. These discrepancies between studies may reflect differences in experimental methods as well as the target cell population. The most striking difference between PB- and CB-derived CD34$^+$ cells is expression of the IL-6R. We found that 80% of the PB-derived CD34$^+$ cells expressed the IL-6R, whereas only 20% of CB-derived CD34$^+$ cells express this receptor (Sakabe et al, unpublished data).

Broudy et al[34] recently reported that TPO-induced colony growth and nuclear maturation of CFU-Meg were not blocked by the addition of neutralizing anti-gp130 MoAb. In contrast, IL-3–induced colony growth and nuclear maturation of CFU-Meg were impaired by the same neutralizing MoAb. These results suggest that signals induced by IL-3 are mediated in part through gp130. It was reported that c-kit antisense oligonucleotides significantly inhibited colony formation by BM-derived CD34$^+$ cells in the presence of IL-3.[35] In addition, Ratajczak et al[36] reported that c-kit antisense oligonucleotides selectively inhibited erythroid-burst formation induced by IL-3 + Epo. These results suggest that c-kit/SCF system predominantly functions in erythropoiesis in association with IL-3. Our previous report[11] was consistent with these results and further suggest that IL-3 may share a common signal transduction pathway with SCF in erythropoiesis. Collectively, IL-3 signaling may function as a key signal for both gp130 and c-kit signal transduction pathways.

Recent studies[18,37] have shown that activation of JAK 2 TK is associated with signal transduction of gp130 as well as receptors for terminally acting lineage-specific factors, including Epo, G-CSF, and TPO, suggesting that gp130 and these receptors (Epo-R, G-CSF-R, and c-Mpl) may share a common intracellular signaling pathway. In addition, signals through common β initiated by IL-3 also activated JAK 2 TK,[37] implying that JAK 2 may play an important role in the cross-talk between these cytokines (signals).

The physiologic significance of sIL-6R may be suggested by its presence in human sera.[38,39] In addition, sIL-6R found in sera is biologically active and can bind to IL-6 and stimulate gp130.[17,38] Furthermore, gp130 knock-out mice showed a marked reduction of the number of colony-forming units in spleen (CFU-S), CFU-GM, and BFU-E in fetal livers.[40] In this study, some gp130$^{-/-}$ embryos show anemia, further suggesting the physiologic role of the signal through gp130 in embryonic hematopoiesis. In addition, Bernad et al[41] suggested that the terminal maturation of myeloid as well as erythroid progenitor cells is impaired in IL-6–deficient mice. Their interpretation of the role of IL-6 is consistent with our results. Very recently, it was reported that IL-6/sIL-6R double transgenic mice displayed a marked hepatosplenomegaly caused by an extreme expansion of extramedullary hematopoietic progenitors.[42] In these double transgenic mice, numbers of neutrophilic granulocytes, platelets, and red blood cells markedly increased in the peripheral blood. This study clearly showed that continuous activation of the gp130 signal transducer leads to an effective production of mature blood cells, further demonstrating in vivo role of gp130 signaling.

Alternatively, it was reported that G-CSF and c-Mpl knock-out mice have mature neutrophils and platelets at 20% to 30% and 6% of the levels in wild-type mice.[43,44] In addition, a few primitive erythrocytes are produced by Epo or Epo-R knock out mice, indicating that some erythroid progenitors can proliferate and differentiate in the complete absence of either Epo or Epo-R.[45] It was also reported that a few definitive erythroid cells could terminally differentiate in Epo-R knock-out mice and that the number of fetal liver-derived CFU-E greatly increased when cultured with SCF and spleen cell-conditioned medium (a source of IL-3 and other cytokines).[46] These results suggest that maturation signals through G-CSF-R, Epo-R, and c-Mpl can be replaced by some other signals and that there may be functional redundancy between specific cytokines (signals). In the present study, we showed that simultaneous activation of the three signals induced proliferation and differentiation of trilineage hematopoietic progenitor cells. Signaling through gp130 may mainly induce maturation of these progenitors and this may partly explain why some mature blood cells are found in the lineage-specific factor and/or receptor knock-out mice. However, our data also indicate that the three signals are apparently less effective than physiologic maturation factors

qualitatively as well as quantitatively in vitro. Therefore, they cannot possibly provide quantitatively enough numbers of mature blood cells that are required to maintain steady-state hematopoiesis in the absence of physiologic maturation factors.

From the other point of view, it was reported that SCF, IL-6, and sIL-6R are detectable in human sera.[38,39] In contrast, IL-3 is only produced by activated T cells and mast cells[47,48] and is not detectable in human serum. Taken together, there is a possibility that the three signals cannot function in normal physiologic hematopoiesis in vivo.

In conclusion, the present study provides support for a new concept that terminal maturation of hematopoietic progenitors may be achieved by simultaneous activation of signals through gp130, c-kit, and IL-3R in vitro. It is still uncertain whether there is a cross-talk between the three signals. However, Wu et al[49] recently reported that SCF induced tyrosine phosphorylation of Epo-R and suggested that the signaling through c-kit may activate the Epo/Epo-R signal transduction pathway. More recently, they clearly showed that a functional interaction between c-kit and the Epo-R is essential for the function of CFU-E progenitors.[50] It was also reported that various chimeric receptors composed of extracellular domains of Epo-R and cytoplasmic domains of IL-2 or IL-3 receptors can induce erythroid-specific gene expression in nonerythroid IL-3–dependent pro-B cells (Ba/F3 cells), suggesting that the cytoplasmic domains of the IL-3R can induce the expression of erythroid-specific gene, including globin, GATA-1, and SCL.[51] Collectively, activation of three signals may lead to cross-activation of additional signals that induce the terminal maturation of hematopoietic progenitor cells. Recent molecular biologic analysis of signal transduction of various cytokines[18] (including IL-6, SCF, and IL-3) may provide a better understanding of the interactions among signals through gp130 and specific cytokine receptors and/or receptor type TKs.

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Cellular and molecular characterization of the role of the FLK-2/FLT-3 receptor tyrosine kinase in hematopoietic stem cells.
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ABSTRACT: The flk-2/flt-3 receptor tyrosine kinase was cloned from a hematopoietic stem cell population and is considered to play a potential role in the developmental fate of the stem cell. Using antibodies derived against the extracellular domain of the receptor, we show that stem cells from both murine fetal liver and bone marrow can express flk-2/flt-3. However, in both these tissues, there are stem cell populations that do not express the receptor. Cell cycle analysis shows that stem cells that do not express the receptor have a greater percentage of the population in G-0 when compared with the flk-2/flt-3-positive population. Development of **agonist antibodies** to the receptor shows a proliferative role for the receptor in stem cell populations. Stimulation with an **agonist antibody** gives rise to an expansion of both myeloid and lymphoid cells and this effect is enhanced by the addition of kit ligand. These studies serve to further illustrate the importance of the flk-2/flt-3 receptor in the regulation of the hematopoietic stem cell.

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The %Mpl% receptor (%Mpl% -R) is a cytokine
AB, NOT AGONIST

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The Mpl Receptor Is Expressed in the Megakaryocytic Lineage From Late Progenitors to Platelets

By Najet Debili, Françoise Wendling, David Cosman, Monique Titeux, Christina Florindo, Isabelle Dusanter-Fourt, Ken Schooley, Nassia Methia, Martine Charon, Roland Nador, Ali Bettaieb, and William Vainchenker

The Mpl receptor (Mpl-R) is a cytokine receptor belonging to the hematopoietin receptor superfamily for which a ligand has been recently characterized. To study the lineage distribution of Mpl-R in normal hematopoietic cells, we developed a monoclonal antibody (designated M1 MoAb) by immunizing mice with a soluble form of the human Mpl-R protein. With few exceptions, Mpl-R was detected by indirect immunofluorescent analysis on all human leukemic hematopoietic cell lines with pluripotential and megakaryocytic phenotypes, but not on other cell lines. By immunoprecipitation and immunoblotting, M1 MoAb recognized a band at 82 to 84 kD corresponding to the expected size of the glycosylated receptor. Among normal hematopoietic cells, M1 MoAb strongly stained megakaryocytes (MK) and Mpl-R was detected on platelets by indirect immunofluorescence staining or immunoblotting. On purified CD34$^+$ cells, less than 2% of the population was stained, but the labeling was weak and just above the threshold of detection. However, dual-labeling with the M1 and antiplatelet glycoprotein MoAbs showed that most Mpl-R$^+$/CD34$^+$ cells coexpressed CD41a, CD61, or CD42a, suggesting that cell surface appearance of Mpl-R and platelet glycoproteins could be coordinated. M1-positive and M1-negative subsets were sorted from purified CD34$^+$ cell populations. Colony assays showed that the absolute number of hematopoietic progenitors was extremely low and no primitive progenitors were present in the CD34$^+$/Mpl-R$^+$ fraction. However, this cell fraction was significantly enriched in low proliferative colony-forming units-MK. When the CD34$^+$/Mpl-R$^+$ fraction was grown in liquid culture containing human aplastic serum and a combination of growth factors, mature MK were seen as early as day 4, whereas the predominant cell population was erythroblasts on day 8. Similar data were also obtained with the CD34$^+$/Mpl-R$^-$ fraction with, however, a delay in the time of appearance of both MK and erythroblasts. In conclusion, Mpl-R is a cytokine receptor restricted to the MK cell lineage. Its expression is low on CD34$^+$ cells and these cells mainly correspond to late MK progenitors and transitional cells. These data indicate that the action of the Mpl-R ligand might predominate during the late stages of human MK differentiation.

PRODUCTION OF blood cells is regulated by specific hematopoietic growth factors acting on survival, proliferation, and differentiation. These cytokines exert their biologic effects through the binding of specific receptors on the cell surface. Most cytokine receptors are members of a large family characterized by a common structural motif within the extracellular domain, containing four conserved cysteine residues and a short tryptophan-serine-X-tryptophan-serine (WSXWS) motif located proximal to the transmembrane region. These receptors do not contain consensus sequences for catalytic activities within the cytoplasmic domain.[1]

The murine myeloproliferative leukemia virus (MPLV) contains a truncated form (*v-mpl*) of the coding region of the *c-mpl* gene that is a member of the hematopoietin receptor superfamily.[2] This mutant virus was isolated from a mouse originally infected at birth with a Friend helper virus.[3] It was shown that the oncogene *v-mpl* transforms, in vivo and in vitro, murine multipotential and committed progenitors leading to the generation of various immortalized hematopoietic cell lines capable of spontaneous differentiation.[2,4,5] cDNAs encoding the human and murine *c-mpl* proto-oncogenes were isolated and sequence analyses showed that *c-mpl* had strong homology to members of the cytokine receptor superfamily.[6-8] Using chimeric receptor constructs, it was shown that the cytoplasmic portion of *c-mpl* contained the elements necessary to transduce a proliferative signal in murine hematopoietic cells.[6,8]

Several lines of evidence strongly suggested that the *c-mpl* product was the receptor of a cytokine specifically involved in the regulation of the megakaryocytic lineage. *c-mpl* transcripts were detected by reverse transcription-polymerase chain reaction (RT-PCR) analyses in human leukemic cell lines with a megakaryocytic phenotype, in megakaryocytes (MK) and platelets, as well as in CD34$^+$ cells.[9] Addition of *c-mpl* synthetic antisense oligodeoxynucleotides significantly decreased *c-mpl* transcrits in CD34$^+$ cells and resulted in a profound reduction of in vitro colony-forming unit-MK (CFU-MK) colony formation, whereas the development of erythroid (burst-forming unit-erythroid [BFU-E]) and granulocyte-macrophage (CFU-GM) colonies was not impaired.[9] Biologic and molecular characterization of the Mpl ligand (Mpl-L) have just been performed.[10-14]

To further clarify the role of Mpl-R in human hematopoiesis, we developed a monoclonal antibody (M1 MoAb) directed to the extracellular portion of the human receptor. Here, we provide data showing that Mpl-R is a growth factor receptor restricted to the MK lineage of differentiation from late MK progenitors to platelets.

MATERIALS AND METHODS

Cell lines culture. UT-7, Mo-7E, and TF-1 (factor-dependent pluripotent cell lines obtained from Dr Komatsu, Tochigi-ken, Japan,

From INSERM U362, Institut Gustave Roussy, Villejuif, France; Immunex Corp, Seattle, WA; INSERM U363, Hôpital Cochin, Paris, France; and the Centre Départmental de transfusion, Hôpital Henri Mondor, Créteil, France.

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and Dr Drexler, Hannover, Germany)[15-17]; Dami, HEL, LAMA, and AP217 (erythro/megakaryoblastic cell lines[18-20]; AP217 kindly provided by R. Berthier, Grenoble, France); CHRF and ELF 153 (megakaryocytic cell lines)[21,22]; K562 (erythroblastic cell line)[23]; KG1a, HL60, and U 937 (myeloid cell lines); and RPMI and CEM (lymphoid cell lines) were maintained in α-minimum essential medium (α-MEM; GIBCO-BRL, France) and 10% heat-inactivated fetal bovine serum (FBS; Gentech, Les Ulis, France). UT-7, TF-1, and Mo-7E were cultivated with 10 ng/mL recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF). Human recombinant cytokines were gifts from Immunex Corp (Seattle, WA) and Amgen (Thousand Oaks, CA). All media were complemented with L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (250 U/mL).

Generation of a human anti–Mpl-R polyclonal antibody. A recombinant protein was obtained by subcloning a *Sph*I-*Hin*dIII fragment encompassing amino-acids 84-231 of the extracellular domain of the human *c-mpl* cDNA[7] into the pGEX2T vector. The recombinant protein was expressed in bacteria. The purified fusion protein (100 μg) was injected subcutaneously into New Zealand rabbits that were boosted twice intramuscularly at 3-week intervals.

Generation of human anti–Mpl-R MoAb. The N-terminal hematopoietin receptor domain of the human *c-mpl* cDNA, from amino acids 26-284,[7] was amplified by PCR and modified at the N-terminus by the addition of a Flag epitope tag[24] and at the C-terminus by the addition of a termination codon. The mpl domain cDNA was inserted into a yeast expression vector under the control of the ADH-2 promoter as a fusion to the α factor leader for secretion as described.[25] The recombinant Flag-Mpl protein was expressed in yeast and purified from culture supernatants by affinity chromatography using an anti-Flag MoAb as described.[26]

A Balb/c mouse was immunized by six subcutaneous injections of 3 to 10 μg of the purified protein. Hybridomas were generated by standard techniques and supernatants from growth-positive wells were tested for their ability to bind the biotinylated immunogen using an antibody capture assay as previously described.[27] Cells from one positive well were single-cell cloned and used to produce the anti–Mpl-R antibody designated as M1 (*anti-Mpl receptor-1*). M1 was identified as an IgG1 isotope using a typing kit (Sigma Chemical, St Louis, MO). This antibody was then shown to immunoprecipitate ^{35}S-labeled full-length human *c-mpl*–encoded protein from CV-1/EBNA cells[28] transfected with a human *c-mpl* expression vector (data not shown). M1 MoAb was unable to immunoprecipitate murine *c-mpl*–encoded protein under the same conditions (unpublished data).

Immunoprecipitation and immunoblot analysis. Immunoprecipitations and immunoblotting were performed as described.[29] Briefly, 2×10^7 cells were solubilized in a mild lysis buffer (20 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1% NP40, 10% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL pepstatin A, 10 μg/mL leupeptin, 10 μg/mL aprotinin). All reagents were purchased from Sigma. Lysates were incubated with the polyclonal rabbit anti–Mpl-R serum (J2) and immune complexes were collected by incubation with protein A-sepharose beads (Pharmacia Biotech, Saint Quentin Yvelines, France). Samples were subjected to 8% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) under reducing conditions and, when indicated, transferred to nitrocellulose membranes for immunoblotting. Blots were incubated with the M1 MoAb, washed, and incubated with a horseradish peroxydase-conjugated antimouse antibody (Amersham, Paris, France). Immune complexes were detected by electrochemoluminescence (ECL; Amersham).

For biosynthetic labeling, cells were preincubated for 1 hour at 37°C in culture medium without methionine and cysteine, and further incubated for 3 hours in the same medium supplemented with a mixture of L^{35}S-methionine and L^{35}S-cysteine (250 μCi/mL; cell labeling mix; Amersham). After washing, cells were solubilized and immunoprecipitated as described above.

Northern analysis. Total cellular RNAs were prepared by the acid guanidium thiocyanate/phenol/chloroform method.[30] Total RNAs (10 μg) were electrophoresed on 1% agarose gels followed by blotting onto nitrocellulose filters (Hybond C; Amersham). The probe was a *Pvu*II-*Pvu*II fragment, covering the third and fourth exons of the *c-mpl* c-DNA,[31] which was (α-^{32}P)dCTP-labeled using a random priming labeling kit (Rediprime; Amersham) to a specific activity of about 5×10^8 cpm/μg. Hybridization was performed overnight at 42°C. Filters were washed with a solution containing 1× SSPE and 0.1% SDS (1× SSPE: 0.15 mol/L NaCl, 0.01 mol/L NaH_2PO_4, and 0.001 mol/L EDTA) and exposed for 10 days at −80°C with intensifier screens.

RT-PCR analysis. Total cellular RNAs were extracted from a pellet of 5×10^6 cells following the procedure described by Gough.[32] RT-PCR was performed as previously described without modification,[9] except that 1 μg of total RNA was used. 5′ and 3′ oligodeoxynucleotides used to amplify c-mpl and β2-microglobulin transcripts and internal oligonucleotide probes were described.[9]

Isolation of bone marrow cell populations. Bone marrow or blood mononuclear cells (PBL) from normal informed individuals were separated through a Ficoll-Hypaque metrizoate gradient (1.077 g/L; Eurobio, Paris, France). CD34$^+$ cells were recovered from marrow light-density cells (usually 2×10^8 cells) by either the immunomagnetic bead or immunopanning techniques. For the immunomagnetic bead technique, cells were first incubated at 4°C for 30 minutes with the QBEND 10 MoAb (CD34; Immunotech, Lumigny, France) at a dilution of 1/2,500 (3 μg/mL) and then with paramagnetic beads coupled to a goat antibody to mouse IgG (Dynabeads M-450; Dynal, Oslo, Norway) at a bead to target cell ratio of 5 to 1. CD34$^+$ cells were isolated by magnetic separation and detached from the beads by chymopapain treatment (Sigma; 130 U/mL for 10 minutes).[33] The CD34 epitope recognized by QBEND 10 is cleaved by chymopapain. This procedure allows the collection of CD34$^+$ cells free from beads and an immediate relabeling of the cells with MoAbs recognizing chymopapain-resistant epitopes on the CD34 molecule. A commercial immunopanning technique (AIS; Techgen, Les Ulis, France) was also used according to the manufacturer's instructions. It includes two steps of purification, i.e., isolation of soybean agglutinin (SBA)-negative cells followed by the isolation of CD34$^+$ cells from the SBA-negative population.

Platelets were isolated from the blood by differential centrifugation and contaminating leukocytes were eliminated by filtration.

To obtain a highly enriched population of human megakaryocytes, total bone marrow cells were cultured in liquid suspension cultures containing Iscove's Modified Dulbecco's Medium (IMDM; GIBCO BRL), 10% serum from human aplastic patients (AP), and 1% deionized bovine serum albumin (BSA; fraction Cohn V; Sigma), as previously described.[34] Cells were washed and usually serum-depleted before being analyzed.

In addition, in a patient with an M7 leukemia, blood samples containing more than 75% CD41$^+$ blast cells were collected and deep frozen for subsequent biochemical analysis.

Antibodies and flow cytometric analysis. Several MoAbs were used for flow cytometry. Fluorescein isothiocyanate (FITC)-conjugated HPCA 2 (8G12, anti-CD34), Beb 1 (anti-CD42a), IOA71 (YDJ1.2.2., anti-CD71), IOB6 (T16, anti-CD38), Dako IIIA (Y2-51, anti-CD61), and anti-CD15 were purchased from Becton Dickinson Monoclonal Center (Mountain View, CA), Immunotech (Lumigny, France), and Dako (Glostrup, Denmark), respectively. Phycoerythrin (R-PE)-conjugated anti-Leu-12 (4G7, anti-CD19) and anti–Leu-M3

(anti-CD14) were purchased from Becton Dickinson. Control isotype antibodies (IgG1, IgG2a, FITC-IgG1, and PE-IgG1) were obtained from Dako. FITC- or PE-labeled sheep antimouse antiserum or IgG F(ab)$'_2$ fragments were obtained from Silenius (Hawthorn, Australia) and Dako, respectively.

Briefly, cells were incubated with M1 MoAb (purified IgG1, 236 μg/mL, 1/100 dilution) in phosphate-buffered saline (PBS; pH 7.4) supplemented with 0.1% BSA for 30 minutes at 4°C. Cells were washed in PBS and then incubated with 1/100 dilution of FITC- or PE-conjugated sheep antimouse IgG F(ab)$'_2$ fragments for 30 minutes at 4°C. The negative control for M1 MoAb was an unrelated mouse IgG1. Cells were subsequently incubated for 10 minutes at 4°C with mouse serum and washed. Cells were then incubated with a directly conjugated MoAb for 30 minutes at 4°C and washed. 7 Amino-actinomycin-D (1 μg/mL; Sigma) was added just before analysis with a FACsort (Becton Dickinson) to eliminate dead cells.[35]

In some experiments, cells were cytospun onto slides, fixed with methanol, and doubly stained with a polyclonal rabbit antiserum against the von Willebrand factor (vWF; Dako) and M1 MoAb. Antibody binding was revealed by a rhodamin isothiocyanate-conjugated sheep antiserum against rabbit immunoglobulins or FITC-conjugated antimouse IgG F(ab)$'_2$ fragments, respectively.

Cell sorting. Purified CD34$^+$ cells were either stained with M1 MoAb alone or doubly labeled with M1 and the R-PE HPCA 2 (anti-CD34) MoAbs. After one washing, cells were suspended in IMDM at a concentration of 5×10^5 cells/mL and separated by cell sorting. Cells were sorted on an ODAM, ATC 3000 cell sorter (ODAM/Bruker, Wissembourg, France) equipped with an INNOVA 70-4 argon ion laser (Coherent Radiation, Palo Alto, CA) tuned at 488 nm and operating at 500 mW or on a FACsort. For both cytometers, a "morphologic" gate that included 80% of the events and all the CD34$^+$ cells was determined on two-parameter histograms by side scatter (SSC) versus either electric measurement of the cell volume or forward scatter (FSC). Compensation for two-color labeled samples was set up with singly labeled samples. Among the CD34$^+$ population, M1 MoAb positivity or negativity were determined by using cells labeled with the R-PE HPCA 2 and an FITC-labeled irrelevant IgG1 MoAb. Because the expression of Mpl-R was quite low in comparison to the IgG1 control, the M1-positive (Mpl-R$^+$) fraction was contaminated by about 15% of negative cells.

Colony assays and liquid cultures. For colony assays, cells were plated in the fibrin clot culture system as previously described.[36] Culture medium was composed of IMDM containing 10% preselected serum from an aplastic patient, 1% deionized BSA, L-asparagine (20 μg/mL), $CaCl_2$ (28 μg/mL), 10% bovine citrated plasma (BCP; GIBCO BRL), recombinant human interleukin-3 (r-hu IL-3; 100 U/mL), r-hu GM-CSF (2.5 ng/mL), r-hu G-CSF (20 ng/mL), and recombinant human erythropoietin (r-hu Epo; 1 U/mL). Colonies were counted under an inverted microscope after 12 days of incubation. In addition, to accurately identify MK colonies, clots were dried in situ, fixed with methanol, and reacted with the Y2-51 anti-GpIIIa MoAb for 30 minutes. Fixation of Y2-51 MoAb was then shown using mouse 1/100 diluted IgG + IgM F(ab)$'_2$ coupled to alkaline phosphatase (Caltag Lab, San Francisco, CA). Enzymatic activity was detected as described.[9] Clots were counterstained with hematoxylin.

For liquid cultures, CD34$^+$ cells positive for Mpl-R surface expression were sorted and cultured in 24-well plates (Costar, Dutscher, Brumath, France) at a cell concentration of 500 cells/mL of IMDM medium containing 10% preselected serum from an aplastic patient, a combination of growth factors (stem cell factor, Epo, and IL-3) and 1% deionized BSA as described.[37] Analysis of the cell population growing in each well was performed by flow cytometry after labeling with directly conjugated MoAbs against CD61, GPA, CD14, and CD15.

RESULTS

Surface expression of Mpl-R on human leukemic cell lines. A number of human hematopoietic cell lines were examined for Mpl-R display by indirect immunofluorescence using the M1 MoAb and the fluorescent profiles of these cell lines are shown in Fig 1. Binding of M1 was clearly detected in the HEL, Mo-7E, and Dami cell lines. A weaker labeling was observed on UT-7, AP217, and TF-1, whereas CHRF, KU 812, K 562, and KG 1a showed binding that was barely detectable. In contrast, HL 60, U 937, or CEM and Jurkatt (not shown) were consistently negative. In general, these results confirmed the data obtained by RT-PCR (data not shown). However, some differences must be pointed out. HEL[7] and Dami cells, which both expressed high amounts of c-mpl mRNA as detected by Northern analysis, showed the highest Mpl-R expression on their cell surface. In contrast, binding of M1 MoAb to CHRF was variable ranging from the threshold of detection to a clear labeling, whereas mRNA expression was at a similar level to Dami (Fig 2). The intensity of staining on Mo-7E, UT-7, AP217, and TF-1 was also variable from one experiment to another. This variability was not dependent on the culture conditions (plateau or exponentially growing cells) or on the presence of serum or growth factors (data not shown). In K 562 cells, a labeling just over the control IgG1 could be detected in some experiments, whereas no transcript could ever be detected by RT-PCR.

Immunobiochemical characterization of Mpl-R from human leukemic cell lines. Expression of Mpl-R on human leukemic cell lines was further examined by immunoprecipitation from metabolically labeled Dami cells or by immunoprecipitation followed by immunoblotting from unlabeled cells. A rabbit polyclonal antiserum (J2) raised against a portion of the Mpl-R extracellular domain was used for immunoprecipitation and M1 MoAb for immunoblotting. As shown in Fig 3, Mpl-R appeared as a protein of approximately 82 to 84 kD that was recognized by both the polyclonal and monoclonal anti-Mpl-R antibodies in lysates from metabolically labeled (lanes 1 and 2) or unlabeled cells (lanes 3 and 4). When analyzing various cell lines, we observed that Dami cells expressed high amounts of Mpl-R protein. The amount of Mpl-R protein was weak in HEL, Mo-7E, and CHRF, low in UT-7, and undetectable in TF-1 or AP 217 (data not shown). These results are in partial agreement with the data obtained from indirect immunofluorescence as exemplified by AP 217 or TF-1 cells, which showed weak surface staining (Fig 1). It is noteworthy that, from cell lines to cell lines, the size of the Mpl-R protein identified by the M1 MoAb varied from 84 kD (Mo-7E and UT-7) to 70 kD (HEL) or appeared as a doublet at 84 kD and 74 kD in the CHRF cell line. These bands could correspond to multiple forms of Mpl-R such as those detected by the cloning data.[6-8]

Mpl-R expression on normal hematopoietic cells. To examine the distribution of Mpl-R among bone marrow cells, light-density mononuclear cells were labeled with M1

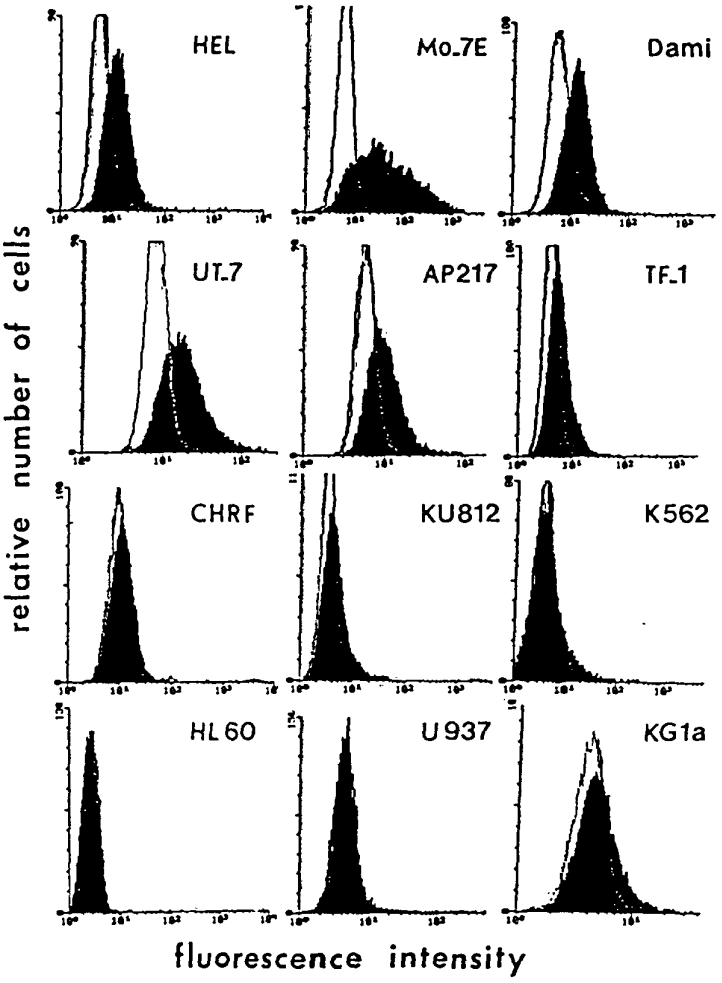

Fig 1. Mpl receptor expression on human leukemic cell lines. Fluorescent profile of representative cell lines. The cells were labeled with M1 MoAb (black profile) or with a control IgG1 (thin line) and indirectly stained with FITC-conjugated sheep anti-mouse IgG F(ab)$_2$ fragments. Cytofluorographs were obtained on a FACsort.

MoAb. In the different scatter gates for granulocytic cells, blast cells, and lymphocytes, no staining distinguishable from the background level of the nonrelevant IgG1 control was observed, with the exception of very rare cells (<0.1%). Mpl-R staining of cells with high scatter properties, which could correspond to polyploid MK, was not investigated because these cells were too rare in marrow samples. No significant labeling was detectable on mononuclear cells from peripheral blood.

Because we have previously shown that c-mpl transcripts were detected by RT-PCR in CD34$^+$ cell populations, MK, and platelets,[9] we focused our studies on these three populations. To determine whether the Mpl-R protein could be seen at the cell surface of MK, bone marrow cells were cultured in liquid in the presence of human aplastic serum to obtain a culture highly enriched in MK (up to 10%). Cells were dually labeled with a PE-conjugated anti-CD61 MoAb and M1 MoAb indirectly labeled with FITC. Staining was analyzed by flow cytometry. The majority of CD61$^+$ cells were stained by M1 (Fig 4). However, some CD61$^-$ cells also appeared to be weakly stained with M1. This was caused by a nonspecific reactivity of M1 MoAb on dead cells, as shown by the addition of 7-amino-actinomycin D to the medium. Among MK, those with large forward and side scatters properties (which corresponded to MK with a high ploidy level) exhibited the strongest staining. Cells from these liquid cultures were also cytospun onto slides and smears were dually labeled with M1 MoAb and a polyclonal anti-vWF antibody. All MK were strongly stained with both antibodies, whereas no significant M1 labeling was seen on cells unlabeled with the anti-vWF antibody (data not shown).

Next, Mpl-R expression was investigated on freshly isolated platelets by both indirect immunofluorescence and immunoblotting. Figure 5 shows that M1 MoAb binds to the Mpl-R expressed on the platelet surface. Fluorescence was much weaker than that observed with an anti-CD61 MoAb, but clearly higher than the background level (Fig 5A). By immunoblotting, M1 MoAb recognized a protein of expected size (82 kD) in a platelet lysate (Fig 5B). The same band but much fainter was also found in lysate from M7 leukemia cells (Fig 5B, lane 3) but not from normal PBL (Fig 5B, lane 2). In addition, in some but not all experiments, a 150-

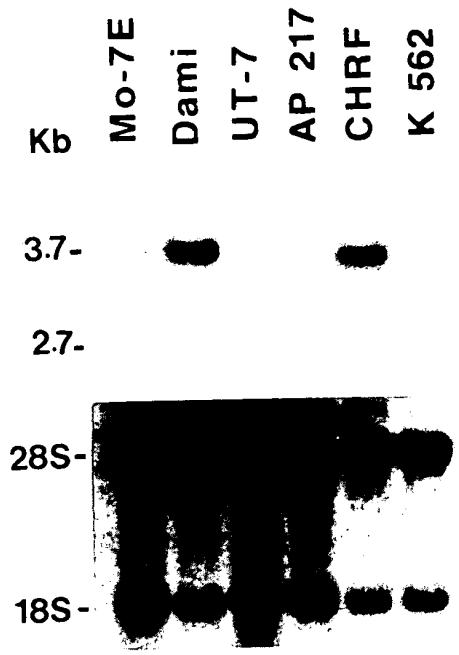

Fig 2. Northern analysis of c-mpl in leukemic cell lines. Total cellular RNA (10 μg) was size separated by formaldehyde/agarose gel and stained with ethidium bromide to visualize 28S and 18S ribosomal RNA. Nitrocellulose filter was hybridized with a human Mpl-R probe. Bands at 3.7 kb are the major c-mpl mRNA. A faint band at 2.7 kb is also seen in Dami and CHRF.[7] Autoradiogram corresponds to a 10-day exposure.

to 160-kD band was also detected in platelet lysates that may correspond to a dimer. Together, these data indicate that both MK and platelets display a detectable number of Mpl receptor molecules on their cell surface.

Mpl-R expression on CD34⁺ cells. Mpl receptor display was then studied on $CD34^+$ cells that are recovered with a purity of about 90% (Fig 6) when either immunomagnetic bead or immunopanning is used. However, the percentage of $CD34^+$ cells stained with M1 MoAb averaged 0.5%.

Mpl-R expression was next analyzed on subsets of $CD34^+$ cells purified by immunomagnetic beads, because chymopapain treatment helps to eliminate most platelets or platelet fragments attached to $CD34^+$ cells. To determine whether $CD34^+/Mpl\text{-}R^+$ cells coexpressed platelet glycoproteins, two-color flow cytometry analyses were performed using $CD34^+$ cells labeled with either FITC-conjugated CD41a (anti-GpIIb), CD42a (anti-GpIX), or CD61 (anti-GpIIIa) and R-PE–labeled M1. Results showed that the vast majority (>80%) of the $CD34^+/M1^+$ cells coexpressed the platelet glycoproteins CD41a (Fig 6) or CD61 (not shown). Nevertheless, a significant fraction of $CD41a^+$ or $CD61^+$ cells were not labeled with M1 MoAb. In contrast, all $CD42a^+$ cells were $M1^+$. However, because the percentage of $CD42a^+$ cells in the $CD34^+$ population was very low, a large proportion of the $M1^+$ cells were in the $CD42a^-$ fraction. These data indicate that the expression of Mpl-R on $CD34^+$ cells appears to be coordinated with the appearance of GpIIb/IIIa. No labeling with M1 was observed on the $CD34^+/CD19^+$ and $CD34^+/CD13^+$ cell populations (data not shown).

Cell sorting. Several cell sorting experiments (n = 14) were performed using $CD34^+$ cell populations. Clonogenic assays and liquid cultures were used to determine the number and nature of progenitors contained in the $CD34^+/M1^+$ or $CD34^+/M1^-$ subpopulations. In the $CD34^+/M1^+$ cell fraction, the absolute number of day-12 clonogenic progenitors (BFU-E, CFU-GM, and CFU-MK) was extremely low and reproducibly less than 2% of the total number of hematopoietic progenitors contained in the initial unseparated $CD34^+$ cell populations (Fig 7A). No primitive progenitors giving rise to colonies within 20 days in culture were present in this cellular fraction. When analyzed in detail, the number of CFU-MK–derived colonies containing 3 or more MK was twofold to threefold increased in the $CD34^+/M1^+$ fraction as compared with the $CD34^+/M1^-$ fraction (Fig 7B). However, the majority of these MK colonies was of small size (range, 3 to 10 MK/colony). In addition, in all experiments, we observed a marked enrichment in individual MK and clusters composed of only 2 or 3 MK. Notably, the ratio between BFU-E and CFU-GM appeared to be slightly inverted in the $CD34^+/M1^+$ fraction when compared with the $CD34^+/M1^-$ or unfractionated $CD34^+$ cells (Fig 7B). Because the $CD34^+/M1^+$ fraction could be contaminated by $M1^-$ cells, the rare cells (<0.1% of the $CD34^+$) that were stained by the control nonrelevant IgG1 were sorted and plated in similar semisolid culture conditions. No isolated MK, MK clusters, or MK colonies were observed. The only contaminant progenitors were rare CFU-GM. As controls, cell sorting was also performed in parallel using $CD34^+$ cells labeled either with anti-CD41a or anti-CD61 MoAbs. A positive staining was clearly seen on a subset (about 3%) of the cells. These positive cells were sorted and grown in culture. Within 4 to 8 days, they gave rise to a nearly pure population of MK clusters (2 to 3 MK) and MK colonies composed of up to 8 cells.

$CD34^+/M1^+$ cell fractions were also cultured for 12 days in liquid culture containing aplastic plasma and a combination of growth factors (r-hu IL-3, 100 U/mL; r-hu GM-CSF, 2.5 ng/mL; r-hu SCF, 20 ng/mL, and r-hu Epo, 1 U/mL). At various times, cells were phenotyped by flow cytometry after been labeled with anti-CD41a, anti-glycophorin A, anti-CD14, and anti-CD15 MoAbs. Mature MK were detected as soon as day 4 in these cultures. At later times, the number of erythroblasts increased greatly and became the predominant population (>90%) on day 8. Similar data were obtained with the $CD34^+/M1^-$ fraction with, however, two main differences: MK appeared later (day 8) and erythroblasts were also the main population at the end of the cultures, but glycophorin A was detectable only after day 10. These results suggest that the $CD34^+/M1^+$ cell fraction may have been slightly enriched in late erythroid progenitors.

DISCUSSION

In this study, we have used a novel MoAb called M1 directed to the extracellular domain of the human Mpl recep-

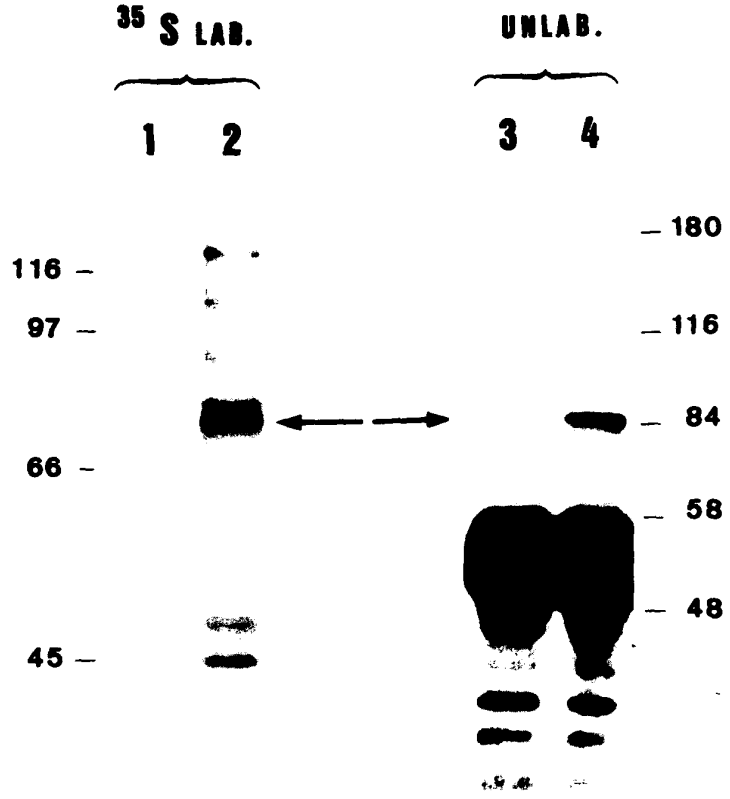

Fig 3. Immunoprecipitation of Mpl-R from a human leukemic cell line. Dami cells (2×10^7) were incubated in the presence (lanes 1 and 2) or the absence (lanes 3 and 4) of ^{35}S-methionine/^{35}S-cysteine for a few hours and lysed. Extracts were immunoprecipitated with a preimmune (lanes 1 and 3) or immune (lanes 2 and 4) anti-Mpl-R rabbit polyclonal antiserum. Immunoprecipitated Mpl-R was detected by autoradiography (lanes 1 and 2) or immunoblotting using the M1 MoAb (lanes 3 and 4) and shown by chemoluminescence.

tor (Mpl-R), the c-mpl product, to define the distribution of this growth factor receptor among hematopoietic cells. M1 MoAb recognizes the Mpl-R on leukemic and normal human hematopoietic cells committed to the MK lineage.

With a few exceptions, all studied human leukemic cell lines with an erythroid/megakaryocytic phenotype display the Mpl-R at their cell surface. In contrast, cell lines with erythroid, myeloid, or lymphoid phenotypes were negative with the exception of a KG1a clone, which, in some experiments, showed a low staining with M1 MoAb. It is noteworthy that this KG1a clone expresses a low level of GpIIb/IIIa.[38] The Mpl-R protein was also detected on three pluripotential factor-dependent cell lines (UT-7, TF-1, and Mo-7E). These cell lines might be useful models to characterize the

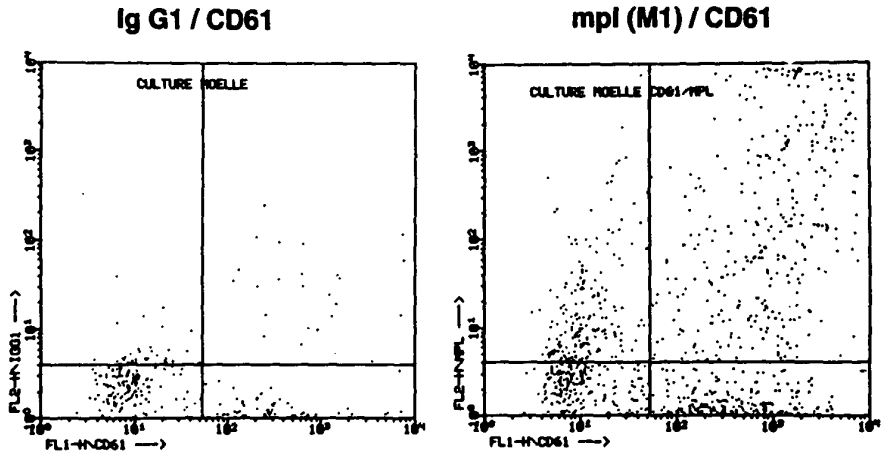

Fig 4. Coexpression of GpIIIa (CD61) and Mpl-R on cultured human MK. Marrow cells were cultured for 12 days in liquid cultures stimulated with a preselected human aplastic serum. Nonadherent cells were labeled simultaneously with a PE-conjugated anti-CD61 MoAb (Y2-51) and either a nonrelevant control IgG1 (left panel) or the specific anti-Mpl-R M1 MoAb (right panel), followed by sheep anti-mouse FITC-conjugated IgG F(ab)$_2$ fragments. The upper right panel indicates cells positive for both GpIIIa and Mpl-R surface expression.

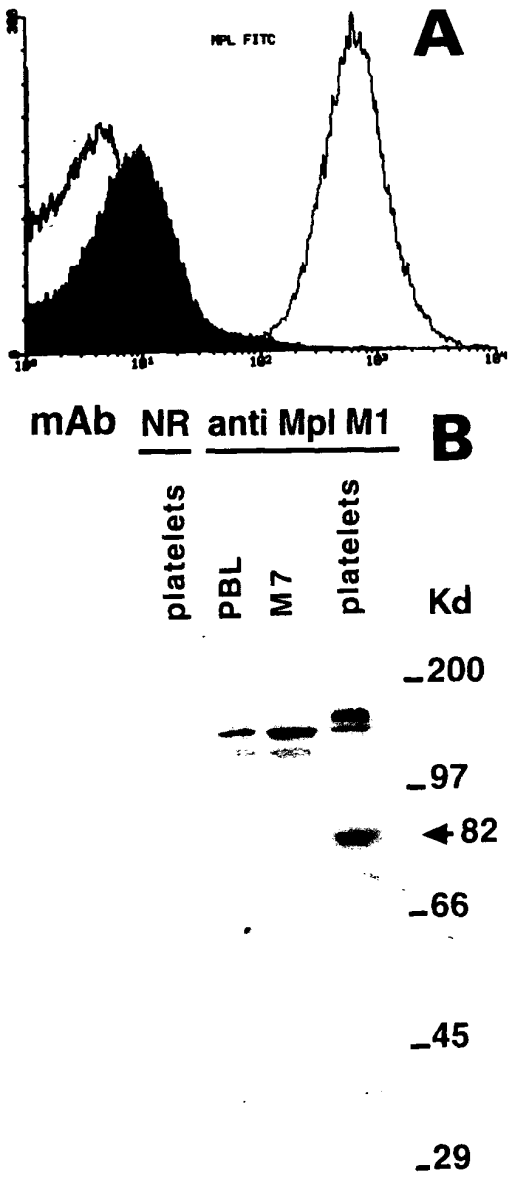

Fig 5. Immunofluorescence staining and immunoblotting of Mpl-R on peripheral blood platelets. (A) Platelets, depleted in leukocytes, were labeled with the anti-Mpl-R M1 MoAb and indirectly stained with FITC-conjugated sheep antimouse IgG F(ab)$_2$ fragments (black profile). Labeling with the control IgG1 is shown by the thick line. The thin line shows the staining with an anti-GpIIIa MoAb. (B) Immunoblotting of Mpl-R in a total platelet lysate shown with M1 MoAb and chemoluminescence. The positions of prestained molecular mass markers are shown on the right. The arrow indicates the molecular mass of the Mpl-R. NR is a nonrelevant IgG1.

effects of the Mpl ligand and to study the transduction signals generated through activation of Mpl-R. Our data are in agreement with prior evaluation using RT-PCR.[9] They indicate that distribution of this growth factor receptor is restricted within the hematopoietic hierarchy. However, we did not find a clear correlation between the cell surface expression of the Mpl-R protein and the amount of mRNA by Northern analysis. For example, CHRF and Dami cells had similar levels of c-mpl transcripts by Northern blot analysis. However, in contrast to Dami cells, binding of M1 MoAb to CHRF was low. Several hypotheses could explain this observation. The human Mpl-R molecule might be heterogeneous, possibly due to different alternate splicing as already described[7,31] and observed for other hematopoietic receptors such as the Epo-R.[39] Thus, the epitope recognized by M1 MoAb may not be present on all Mpl-R molecules. Alternatively, only a fraction of the Mpl-R molecules might be transported to the cell surface, with the remainder retained in the cytoplasm. Further experiments are required to determine the exact processing of Mpl-R and its intracellular trafficking.

The data show that, in normal unseparated hematopoietic cells, Mpl-R was detected by flow cytometric analyses on a very small number of cells (<0.1% of total bone marrow cells) that nearly all belonged to the MK lineage. Noteworthy, cells showing the highest fluorescence intensity in total bone marrow cells were polyploid MK, which were excluded from the gates. The ability to detect Mpl-R on MK was further shown by analysis on marrow cultures enriched in MK where M1 MoAb binding clearly increased in parallel to MK maturation. In agreement with this observation, Mpl-R was also detected on platelets both by immunolabeling and Western blotting. These results on Mpl-R protein expression extend our previous observations obtained at the mRNA level.

To further delineate the developmental and lineage expression of Mpl-R in normal hematopoietic progenitors, subsets of $CD34^+$ cells were analyzed because c-mpl transcripts were previously detected in this cell population. Only a small fraction of $CD34^+$ cells (average, 0.5%) reacted positively and unambiguously with M1 MoAb and their great majority coexpressed the platelet glycoproteins GpIIb/IIIa. No labeling was seen on other $CD34^+$ subsets. However, because both the staining intensity obtained with M1 MoAb and the number of positive cells were low, it remains difficult to exclude that a weak staining might also exist on other $CD34^+$ subsets. The weak labeling observed with M1 MoAb is likely due to the low number of receptor molecules expressed on the surface of $CD34^+$ cells as reported for other cytokine receptors.[40,41]

To sort the $CD34^+$ subsets expressing Mpl-R, we cautiously outlined the windows that gave the clearest separation between $CD34^+$/Mpl-R^+ and $CD34^+$/Mpl-R^- cells. This did not avoid a cross-contamination of about 15%, in comparison to the control IgG1. Nevertheless, colony assays showed that the $CD34^+$/Mpl-R^- fraction contained a number of BFU-E, CFU-GM, and CFU-MK progenitors similar to that found in unseparated $CD34^+$ populations. In contrast, the $CD34^+$/Mpl-R^+ fraction was almost totally depleted in primitive progenitors (<2%) and clearly enriched in late MK progenitors and transitional cells. These MK progenitors had limited proliferative capabilities because they gave rise to MK clusters comprising 2 to 3 MK or individual MK within 5 to 7

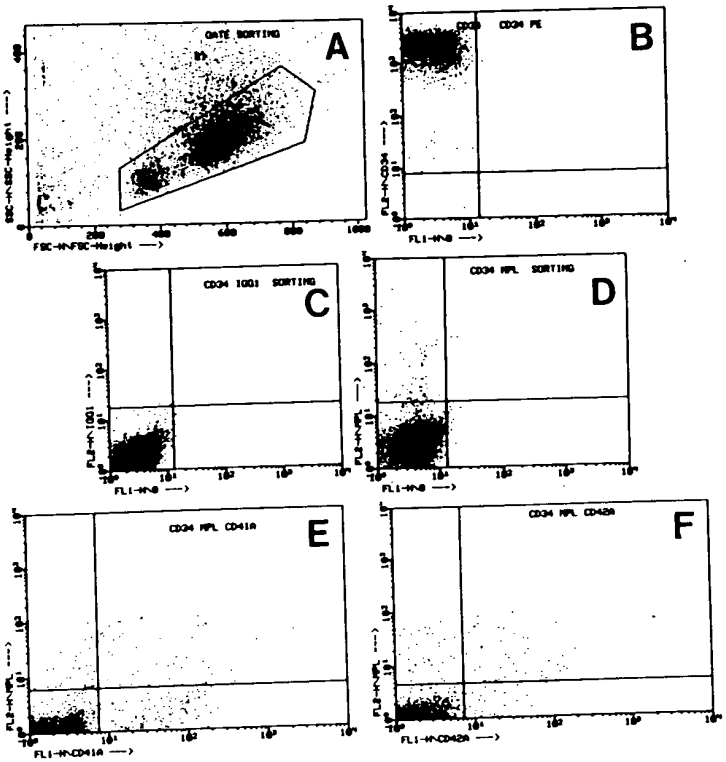

Fig 6. FACS analysis of surface expression of Mpl-R and its coexpression with platelet glycoproteins on purified CD34⁺ cells. CD34⁺ cells were purified by the immunomagnetic beads technique using the QBEND 10 MoAb, detached from the beads by chymopapain, immediately labeled with an R-PE–conjugated anti-CD34 MoAb (8G12), and analyzed on a FACsort. (A) The side scatter (SSC) versus forward scatter (FSC) of the cell population. (B) shows that more than 90% of the gated cells expressed the CD34 antigen. (C) and (D) show the fraction of CD34⁺ cells expressing Mpl-R. Cells were labeled with either a nonrelevant IgG1 (C) or the anti–Mpl-R M1 MoAb (D) and indirectly stained with R-PE–conjugated sheep antimouse F(ab)'₂ fragments. Cells positively stained with M1 MoAb are shown in the upper left rectangle in (D). (E) and (F) illustrate the results of two-color analyses of CD34⁺ cells dually stained with R-PE–labeled M1 MoAb (y axis) and FITC-conjugated anti-GpIIb (CD41a) or anti-GpIX (CD42a) MoAbs (X axis). Cells appearing in the upper right panels are those that coexpressed Mpl-R and platelet glycoproteins.

days in culture. Some true day-12 CFU-MK were also present and about twofold to threefold enriched in some experiments. As reported previously, although controversial,[42,43] CFU-MK progenitors coexpress CD34 and GpIIb/IIIa. We thus examined the CFU-MK progenitor content in the $CD34^+/GpIIb/IIIa^+$ sorted fraction in comparison to that in the $CD34^+/Mpl\text{-}R^+$ subpopulation. A much higher proportion of day-12 CFU-MK was found in the $CD34^+/GpIIb/IIIa^+$ cell fraction. This difference may only be caused by the low level of cell surface Mpl-R expression as compared with GpIIb/IIIa, which likely introduces a skew in the cell sorting. Previously, we have reported that c-mpl transcripts were present in the $CD34^+/CD38^{-/low}$ fraction that has been described to be a cellular fraction highly enriched in primitive progenitors.[44,45] In more recent studies, we found that this fraction is also enriched in MK progenitors, including late CFU-MK.[46] Thus, the presence of c-mpl mRNA in the $CD34^+/CD38^{-/low}$ fraction might be more the reflection of an enrichment in MK progenitors than an expression in primitive hematopoietic cells.

It is noteworthy that, in all experiments, we observed an increase in the relative ratio between mature BFU-E and CFU-GM in the $CD34^+/Mpl\text{-}R^+$ fraction. This enrichment was obvious in liquid cultures because erythroblasts were nearly the only population found after 8 days. Presently, it is difficult to ascertain whether this finding reflects a specific labeling of mature BFU-E by M1 MoAb because only 2% of the total BFU-E number was present in this cellular fraction. Still, when the rare cells (<0.1%) labeled with the nonrelevant IgG1 were sorted and grown in semisolid cultures, almost no BFU-E–derived colonies were observed. Therefore, the data cannot exclude that a subset of BFU-E does express Mpl-R.

Together, our results indicate that expression of Mpl-R seems to follow the same sequence as GpIIb/IIIa during MK differentiation. Interestingly, recent studies on the human c-mpl gene have shown that the structure of its proximal promoter resembles that of GpIIb and other MK-specific genes with both the presence of GATA and ets binding sequences.[31] This finding may explain the restricted expression of the Mpl-R protein to the MK lineage and its coexpression with the platelet glycoproteins IIb/IIIa.

Finally, it is tempting to compare the expression of the Mpl-R to that of the Epo receptor (Epo-R). Both are lineage-restricted receptors, but a promiscuity between the erythroid and megakaryocytic lineages of differentiation is supported by numerous observations. First, the existence of a bipotential E/MK progenitor has been reported.[46-48] Second, the Epo-R is expressed on cells from the MK lineage[49] and we cannot formally exclude the possibility that the Mpl-R is transiently expressed on erythroid progenitors (this report). Third, production of red blood cells and platelets appears to be inversely related.[50-52] Expression of both the Epo-R and Mpl-R predominates during the late stages of differentiation. In particular, it has been shown using fluorescent-labeled Epo that binding was only detected on glycophorin A^+ cells but not on $CD34^+$ cells.[40] Using an MoAb recognizing the Epo-R, we were able to sort immature proerythroblasts and CFU-

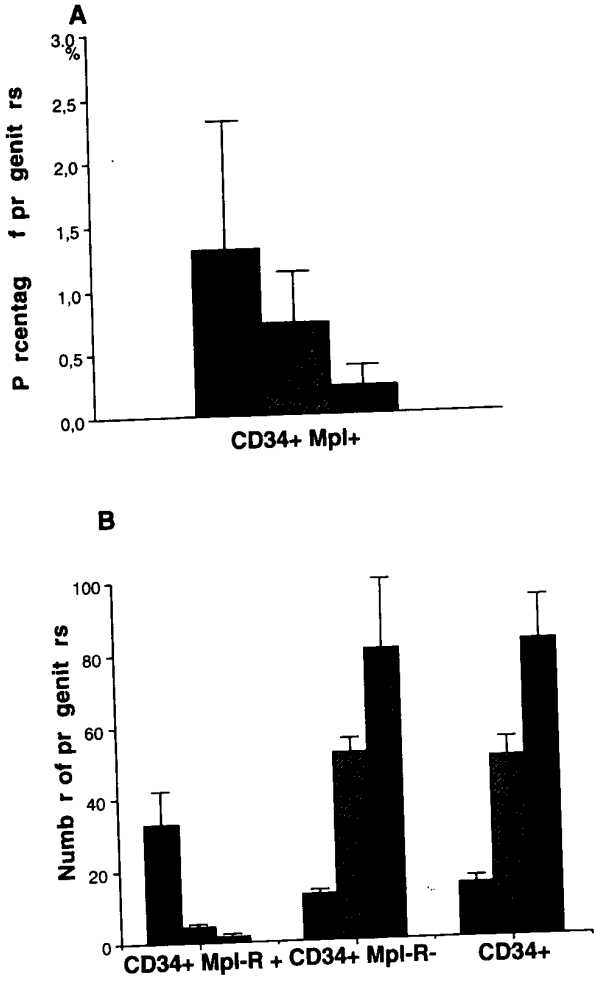

Fig 7. Histograms representing the numbers of progenitors determined by clonogenic semisolid culture assays of $CD34^+/Mpl\text{-}R^+$ or $CD34^+/Mpl\text{-}R^-$ sorted subpopulations. The percentage of (■) CFU-MK, (▨) BFU-E, and (▤) CFU-GM progenitors recovered in the $CD34^+/Mpl\text{-}R^+$ fraction is represented in (A). The number of CFU-MK–, BFU-E–, and CFU-GM–derived colonies in the $CD34^+/Mpl\text{-}R^+$, $CD34^+/Mpl\text{-}R^-$, or unseparated $CD34^+$ fractions are compared in (B). Results represent the mean ± SD of 10 replicate experiments where sorted cells were plated at a concentration of 1,000 cells/mL/dish, with each culture being performed in duplicate.

E to near purity but no BFU-E from normal marrow (personal unpublished data). Similarly, the fluorescence intensity with M1 MoAb is maximal on MK that are already engaged in the endomitotic process of maturation (Fig 4). However, it must be emphasized that the immunolabeling technique has a low-detection sensitivity to investigate hematopoietic growth factor receptor expression.[41] Therefore, the absence of high proliferative CFU-MK and BFU-E in the $Mpl\text{-}R^+$ or $Epo\text{-}R^+$ cell fractions, respectively, does not exclude the presence of these receptors at low levels on primitive progenitors. In favor of this hypothesis, it has been shown that the GM-CSF-R could not be detected on normal CFU-GM by cell sorting.

From the recent experiments that have functionally and molecularly characterized Mpl-L,[10-14] it is clear that Mpl-L is a humoral growth factor restricted to the MK cell lineage that has both the properties of thrombopoietin and MK-CSF. Its biologic activity on the megakaryocytic lineage mimics that of Epo in the erythroid lineage. The present study further strengthens the assumption that the Mpl-R is restricted to the MK lineage and strongly suggests that the action of its ligand will predominate during late stages of MK differentiation.

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Abstract: A heterogeneity in the molecular weight (M-r) of thrombopoietin (TPO) has been reported, We found several thrombin cleavage sites in human, rat, murine, and canine TPOs, and also found that human TPO

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Thrombin cleaves recombinant human thrombopoietin: One of the proteolytic events that generates truncated forms of thrombopoietin

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ABSTRACT **A heterogeneity in the molecular weight (M_r) of thrombopoietin (TPO) has been reported. We found several thrombin cleavage sites in human, rat, murine, and canine TPOs, and also found that human TPO undergoes selective proteolysis by thrombin. Recombinant human TPO (rhTPO) was incubated with human platelets in the presence of calcium ions to allow the generation of thrombin, and was cleaved into low M_r peptide fragments. The cleavage was completely inhibited by hirudin, indicating that the proteolysis was mediated by thrombin. In a platelet-free system, analyses of thrombin cleavage by immunoblotting using anti-human TPO peptide antibodies revealed that the four major thrombin-cleaved peptide fragments were selectively generated depending on the digestion time. The amino acid sequences of the thrombin-polypeptides were further analyzed, and two major thrombin cleavage sites were determined. One of them was at AR^{191}-T^{192} in the C-terminal domain of TPO, and thrombin cleaved first at this site. The other site at GR^{117}-T^{118} in the N-terminal domain was subsequently cleaved by prolonged thrombin digestion. As a result, the biological activity of TPO was modulated. The generation of truncated forms of TPO by thrombin may be a notable event in view of the platelet-related metabolism of TPO.**

Endogenous thrombopoietin (TPO), c-Mpl ligand has an essential role in megakaryopoiesis and platelet production (1). The presence of truncated forms of TPO in thrombocytopenic plasma from various species has been noted by several groups in their studies on the purification of TPO (2–5), and the electrophoretically estimated molecular weight (M_r) of these forms ranged from 18,000 to 36,700. In addition, the heterogeneity in M_r, ranging from 17,000 to 43,000, was also found among native TPOs derived from cell culture supernatants of rat hepatoma cells (6). Although alternative splicing of TPO mRNA may contribute to the heterogeneity of TPO, a multiplicity of biologically active TPOs suggests that the various forms may largely result from proteolysis. The proteolytic activity could be present in the plasma; however, the generation of such various forms of TPO could not arise merely during several stages of purification (7).

Recent findings indicate that circulating platelets may regulate the plasma concentration of TPO (3). We and others have shown that platelets have functional receptors (c-Mpl) on their surface. Thus, TPO enhances the degree of activation of platelets by various agonists or by shear stress, and induces protein tyrosine phosphorylation of numerous proteins in platelets such as Jak2, Tyk2, Shc, c-Cbl, Stat3, Stat5, and Crkl (8–11). Kuter *et al.* (3, 12) have shown that plasma TPO levels increased in Busulfan-treated thrombocytopenic rabbits and that infusion of platelets into the rabbits lowered the plasma levels of TPO. Fielder *et al.* (13) have shown that normal mouse platelets adsorb TPO, whereas platelets from c-mpl −/− mice did not, indicating that the adsorption of TPO by platelets is mediated through the specific TPO receptor (c-Mpl). They also demonstrated that platelet-rich plasma (PRP) but not platelet-poor plasma (PPP) degraded radiolabeled TPO, suggesting that the cleavage is platelet-dependent. To date, no natural protease has been identified to cleave TPO specifically.

A number of potential proteolytic cleavage sites exist in the human TPO. Among them, we found several thrombin cleavage sites (14) in human (2, 15, 16), rat (4, 17), murine (16, 18), and canine (16) TPOs. Thrombin is a multifunctional serine protease acting in the regulation of the coagulation cascade as a key factor, and it also activates various cells such as platelets, smooth muscle cells, and endothelial cells (19). Importantly, guinea-pig megakaryocytes were shown to generate thrombin from prothrombin, and proplatelet formation was affected by thrombin (20). Moreover, thrombin plays functional roles in the regulation of megakaryopoiesis (21). Because thrombin can be formed on the surface of platelets or microparticles derived from platelets, we postulated that thrombin may cleave TPO. In this study, we demonstrate that recombinant human TPO (rhTPO) undergoes selective proteolytic cleavage in the presence of platelets plus calcium ions to allow the generation of thrombin, and that purified thrombin also cleaves TPO. Further, the major cleavage sites in TPO have been determined. These findings may be of significant importance to better understand the metabolism of TPO and the regulation of its plasma level by platelets.

MATERIALS AND METHODS

Proteins. rhTPO was expressed in Chinese hamster ovary cells, and purified to homogeneity by the Production Technology Group of Kirin Brewery Co., Ltd. Purified human plasma thrombin was generously provided by Green Cross (Osaka). Recombinant hirudin was kindly provided by Japan Energy (Tokyo).

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Abbreviations: DPBS, Dulbecco's phosphate buffered saline; ECL, enhanced chemiluminescence; MAP, multiple antigenic peptide; PPP, platelet-poor plasma; PRP, platelet-rich plasma; PVDF, polyvinylidene difluoride; TPO, thrombopoietin; rhTPO, recombinant human TPO.

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Platelet Preparation and Digestion of rhTPO with Platelet Suspension. Human blood from healthy volunteers was drawn by venipuncture into 1/10 volume of 3.8% (wt/vol) trisodium citrate and gently mixed. PRP was prepared by centrifuging the whole blood at 200 × *g* for 20 min and aspirating PRP. Prostaglandin E1 (1 μM; Sigma) was added from a stock solution in absolute ethanol (1 mM). The PRP was centrifuged at 800 × *g* to form a soft platelet pellet. The pellet was resuspended in a modified Hepes·Tyrode buffer (129 mM NaCl/8.9 mM $NaHCO_3$/0.8 mM KH_2PO_4/0.8 mM $MgCl_2$/5.6 mM dextrose/10 mM Hepes, pH 7.4) at a concentration of 3×10^8 platelets per ml. Then, digestion of rhTPO by the platelet suspension was performed as described in detail in the figure legends. Briefly, rhTPO was added to a platelet suspension (3×10^8 platelets per ml in a nominally calcium-free modified Hepes·Tyrode buffer) and incubated for various times at 37°C in the presence or absence of 1 mM calcium ions. EGTA (1 mM) was suitably added to the incubation mixture to block the generation of thrombin by calcium ions. To confirm that the generation of truncated rhTPO was mediated by thrombin, we incubated rhTPO with the platelet suspension in the presence of recombinant hirudin (10 units per ml) plus 1 mM calcium ions. After the incubation, the resultant suspensions were lysed by adding 2× concentrated SDS/PAGE buffer (final concentrations of 10% glycerol/1% SDS/1 mM DTT/50 mM Tris·HCl, pH 6.8/1 mM EDTA/0.002% bromophenol blue), heated for 3 min at 95°C, and then subjected to 7.5–15% gradient SDS/PAGE under reducing conditions. Proteins were visualized by immunoblotting, as described below.

Digestion of rhTPO with Thrombin in Platelet-Free Incubation. rhTPO (50 μg/ml) was incubated in the presence or absence of human thrombin (5 units per ml) in Dulbecco's phosphate buffered saline (DPBS; Nissui Pharmaceuticals, Tokyo) at 37°C for various periods as indicated in the figure legends. The proteolytic reaction was terminated by the addition of one-fourth volume of 5× concentrated SDS/PAGE buffer followed by heat treatment for 3 min at 95°C. The aliquot was subjected to SDS/PAGE for silver staining or Western blotting.

Antibodies to TPO and Synthesized TPO Peptides. Anti-rhTPO rabbit IgG fraction (anti-rhTPO Ab) was obtained from antiserum (22) by use of a Protein A Hyper D column (BioSepra, Marlborough, MA). To prepare anti-TPO peptide rabbit antibodies (anti-TPO peptide Abs), we selected six peptide regions, D^{8}LRVLSKLLRDSHVLHSRLSQ^{28} (HT1), S^{47}LGEWKTQMEETKAQD^{62} (HT2), L^{108}GTQLPPQGRTTAHKDPNA^{126} (HT3), N^{172}ELPNRTSGLLETNFTASA^{190} (HT4), S^{262}LPPNLQPGYSPSPTHPPTGQYT^{284} (HT5), and P^{306}SAPTPTPTSPLLNTSYTHSQNLSQEG^{332} (HT6), as suitable peptide antigens from the amino acid sequence of TPO. Then, each quadruple-stranded multiple antigenic peptide (MAP) was synthesized by a model 431A peptide synthesizer (Perkin–Elmer) by the procedure of Tam (23). Rabbits were then immunized eight times with 100 μg of a given MAP, and antisera were collected. Each anti-TPO peptide Ab was purified by use of a Sulfo-Link affinity column (Pierce), to which a Cys residue at the C terminus of the monomeric peptide was coupled. In brief, a solution of each monomeric peptide was bonded via a Cys residue during a 15-min incubation to Sulfo-Link coupling gel previously equilibrated with the coupling buffer (5 mM EDTA/50 mM Tris·HCl, pH 8.5). Then, the gel was washed with the same buffer, and 0.05 M L-Cys-HCl was added for blocking over a 15-min period. The antiserum was applied to the peptide antigen column pre-equilibrated with 50 mM phosphate buffer (pH 8.0) containing 150 mM NaCl and 0.05% sodium azide, and washed with the same buffer. The adsorbed monomeric antigen peptide-specific anti-TPO peptide Ab was then eluted with 0.1 mM citrate buffer (pH 3.0) and neutralized with a 0.1 M carbonate buffer (pH 9.9). The coupling reactions and purification were carried out at 25°C.

Gel Electrophoreses and Western Blot Analyses. The reduced samples prepared in a SDS/PAGE buffer were subjected to SDS/PAGE using a 10–20% precast gradient gel (Daiichi Pure Chemicals, Tokyo). Prestained protein markers (New England BioLabs) were used for electrophoretic estimation of M_r. After electrophoresis, the proteins were visualized by silver staining (2D-Silver Stain II, Daiichi Pure Chemicals), or transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore). Protein transfer was carried out for 2 hr at a constant current of 200 mA with a semi-dry electroblotter (model HEP-1, Owl Scientific, Woburn, MA) by using solutions of the anolyte (0.3 M Tris/20% methanol, pH 10.4), the transfer membrane solution (25 mM Tris/20% methanol, pH 10.4), and the catholyte (25 mM Tris/40 mM aminocaproic acid/20% methanol, pH 10.4). The blots were washed with 20 mM Tris·HCl/0.5 M NaCl, pH 7.5 (TBS) containing 0.1% Tween 20 (TTBS) for 10 min, and washed with TBS for 10 min. The blots were then treated with 0.1% $NaIO_4$ for 30 min for removal of sugar chains to obtain maximal detection with each anti-TPO peptide Ab. After blocking with a gelatin hydrolysate (Boehringer Mannheim) in TTBS (GTTBS) for 60 min followed by subsequent blocking with Block Ace (Dainippon Pharmaceutical, Osaka) for 60 min, each blot was incubated for 15 hr with 1 μg/ml of anti-rhTPO or anti-TPO peptide Ab, and then washed twice with TTBS. After that, the blots were incubated for 1 hr with goat anti-rabbit biotinylated IgG (Dako), washed with TTBS twice for 10 min each time, and treated with peroxidase-conjugated streptavidin (Dako) for 1 hr. After five washes with TTBS, the proteins were detected by an enhanced chemiluminescence (ECL) method according to the manufacturer's instructions (Amersham). The Western blot analysis described here was carried out at 25°C except for the first antibody reaction, which was performed at 4°C. All antibodies and peroxidase-conjugated streptavidin were prepared in a mixture solution of Block Ace and GTTBS (1:1).

Cell Proliferation Assay for Measurement of the TPO Activity. To measure *in vitro* TPO activity of thrombin-cleaved rhTPO, we conducted a cell proliferation assay using FDCP-hMpl5 cells (11), which were genetically engineered to constitutively express human c-Mpl, as previously reported (24). Briefly, the thrombin digestion was terminated by the addition of hirudin without a reducing reagent. Each diluted aliquot (0.1 ml) was added to a suspension (0.1 ml) of the exponentially growing FDCP-hMpl5 cells (2.5×10^4 cells per ml), and incubated on a 96-well microplate in Iscove's modified Dulbecco's medium with 10% FCS in 5% CO_2 for 72 hr at 37°C. Thereafter, a 20-μl mixture of a tetrazolium compound [3-(4,5-dimethylthiozol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS)] and phenazine methosulfate as a coupling reagent (CellTiter 96 AQ Assay, Promega) was added into each well and the cells were further incubated for 4 hr at 37°C. The cell growth was determined by measuring the absorbance at 492 nm.

Identification of the Thrombin-Cleaved Peptide Fragments. For preparation of a sufficient amount of thrombin-digested peptide fragments for the amino acid sequence analysis, a higher concentration of rhTPO (900 μg/ml), in comparison with that used in the time course experiments described above, was incubated at 37°C for 16 hr in DPBS in the presence of a concentration of thrombin (9 units per ml). The resultant aliquot was analyzed on SDS/PAGE, and showed the identical cleavage profile to that obtained by incubation of rhTPO (50 μg/ml) with thrombin (5 units per ml) for 16 hr at 37°C as shown in Figs. 2 and 3. The major thrombin-cleaved peptide fragments were transferred onto a PVDF membrane after SDS/PAGE was stained with Coomassie brilliant blue R-250, and subsequently destained with methanol before the analysis. Thereafter, each thrombin-cleaved peptide fragment was sub-

jected to Edman degradation followed by amino acid sequencing with a model 492 gas-phase protein sequencer (Perkin–Elmer) equipped with an on-line phenylthiohydantoin amino acid analyzer. The amino acid sequence consisting of more than 20 amino acid residues from the N-terminal end of each fragment was determined. Peptide fragments were also subjected to an amino acid composition analysis to determine the C-terminal end by the AccQ Tag amino acid analysis system using a fluorescent derivatizing reagent (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, Millipore, Waters; ref. 25).

RESULTS

Human Platelet Suspensions Cleave rhTPO. In view of the recent report that murine TPO is cleaved by PRP but not by PPP (13), we first examined whether human platelets also cleave rhTPO. PRP was centrifuged once to remove a portion of plasma, and the platelets were resuspended in a buffer containing 1 mM $CaCl_2$ and exogenous rhTPO. There was a time-dependent accumulation of a 34-kDa protein and another weak diffuse band (46–69 kDa) recognized by the anti-rhTPO Ab (Fig. 1*A*). Under the conditions, there was a significant contamination by plasma proteins, including prothrombin, in the platelet suspensions. Accordingly, we suspected that rhTPO is cleaved by thrombin generated on the surface of platelets in the presence of calcium ions and that the 34-kDa protein represents the cleaved peptide fragment of rhTPO. To directly test this possibility, rhTPO was incubated with platelet suspensions in the presence or absence of calcium ions, and we found that the 34-kDa protein appeared in the presence of 1 mM Ca^{2+} (Fig. 1*B*). Even in the presence of 1 mM Ca^{2+}, the appearance of the 34-kDa and other diffuse TPO-associated proteins was inhibited by the addition of hirudin, a specific inhibitor of thrombin (26–28). These proteins corresponded to peptide fragments generated by the incubation of rhTPO with purified thrombin (Fig. 1*B*). Thus, it is likely that platelet suspensions cleave rhTPO and that the cleavage is mediated by thrombin.

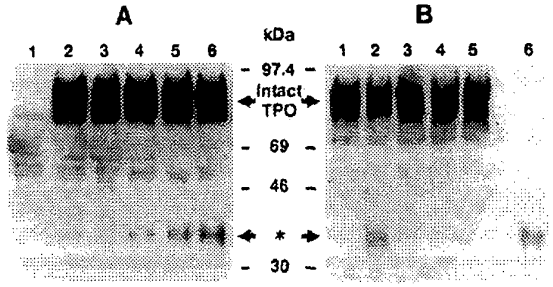

FIG. 1. Cleavage of rhTPO by platelet suspensions. (*A*) Time course. Platelet pellets were resuspended in a nominally calcium-free modified Hepes·Tyrode buffer at a concentration of 3×10^8 platelets per ml. RhTPO (1 μg/ml) and 1 mM calcium ions were or were not added to the platelet suspension, and incubation was conducted for various times at 37°C. The platelets were then lysed by the addition of 2× concentrated SDS/PAGE buffer, and subjected to 7.5–15% gradient SDS/PAGE. Proteins were then electroblotted onto a PVDF membrane, stained by anti-rhTPO Ab, and detected by the ECL method, as described. Prestained protein markers were obtained from Amersham. Lanes: 1, platelet lysate only without exogenous TPO; 2, sample immediately lysed after the addition of rhTPO (1 μg/ml) and 1 mM calcium ions; 3–6, samples lysed 1, 10, 30, and 60 min, respectively, after those additions. The position of the intact rhTPO and the 34-kDa protein band of interest (*) are indicated by the arrows. (*B*) The cleavage of rhTPO is inhibited by hirudin and requires calcium ions. Platelets were lysed by the addition of 2× concentrated SDS/PAGE buffer immediately or after incubation for 1 hr at 37°C with rhTPO (1 μg/ml) in the presence or absence of 1 mM calcium ions. Lysed samples were subjected to 7.5–15% gradient SDS/PAGE. RhTPO was detected as described in *A*. Lanes: 1, immediately lysed sample; 2, 1-hr incubation in the presence of calcium ion (1 mM); 3, 1-hr incubation without the addition of calcium ions; 4, 1-hr incubation with EGTA (1 mM); 5, 1-hr incubation in the presence of calcium ion plus hirudin (10 units per ml); 6, free rhTPO incubated with thrombin (10 units per ml).

Transition of Thrombin-Generated Polypeptides Derived from rhTPO and *in Vitro* TPO Activity. To further characterize the cleavage of rhTPO by thrombin, we examined the fragmentation of rhTPO by exogenous human thrombin in platelet-free systems. In preliminary experiments, we observed by SDS/PAGE that thrombin digestion generated various peptide fragments. Therefore, the first experiment was performed to determine whether such generation of multiple peptide fragments was dependent on the digestion time. The digestion was performed for periods of from 0 to 64 hr. To compare relative contents of peptide fragments on SDS/PAGE, we conducted silver staining for nonspecific protein detection separately from immunochemical staining (Fig. 2*A*). The incubation of full-length rhTPO (intact rhTPO) in the presence of thrombin resulted in the generation of multiple peptide fragments, and the content of each of these polypeptides changed depending on the incubation period. A 34-kDa protein appeared within 0.5 hr, as was found by the incubation of rhTPO with platelets. Then, the maximal detection was obtained after around 4 hr of incubation, and gradually decreased in a time-dependent manner. At 64 hr of incubation, silver-detectable intact rhTPO was almost absent. The thrombin-cleaved peptide fragments were more clearly visualized on immunoblots probed with anti rhTPO Ab, as shown in Fig. 2*B*. The major thrombin-cleaved peptide fragments were designated as FA (around 48 kDa), FB (around 34 kDa), FC (around 25 kDa), and FD (below 10 kDa). Both of the FA and FB appeared within 0.5 hr; the FA gradually increased, whereas the FB decreased. In addition, the much lower M_r peptide fragments (FC and FD) became detectable after 4 or more hr of incubation. The appearance of FC and FD corresponded to the reduction in the amount of FB. The generation of such peptide fragments was completely inhibited by the

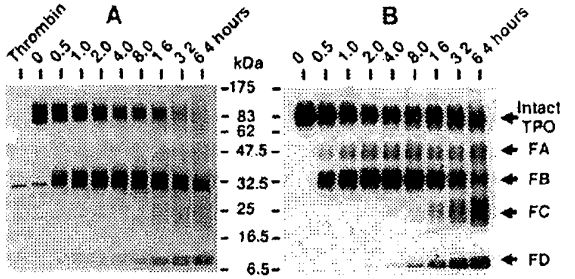

FIG. 2. Time course analysis of thrombin cleavage of rhTPO in a platelet-free incubation. Thrombin cleavage of rhTPO was analyzed by SDS/PAGE under reducing conditions. Proteins were visualized by silver staining for nonspecific protein detection (*A*), and also by immunoblotting using anti-rhTPO Ab (*B*). To examine the time-dependent transition of the formation of thrombin-digested components, rhTPO (50 μg/ml) was incubated at 37°C in the presence of thrombin (5 units per ml). The reaction was terminated at 0.5, 1.0, 2.0, 4.0, 8.0, 16, 32, or 64 hr by the addition of SDS/PAGE sample buffer and heat treatment at 95°C for 3 min. (*A*) A 10-μl aliquot after the digestion containing rhTPO (400 ng) and thrombin (0.04 units) was applied to each lane for silver staining. As a control, thrombin (0.04 units) without rhTPO was charged onto the left lane. The major band (above 30 kDa) in the thrombin preparation was α thrombin. (*B*) A 0.025-μl aliquot containing rhTPO (1 ng) and thrombin (100-μ units) was applied to each lane and detected by the ECL method. The cleavage products, having a M_r lower than that of the intact TPO were classified into four major ones (FA, FB, FC, and FD). These peptide fragments were transferred onto a PVDF membrane, and their N-terminal amino acid sequence and amino acid composition were analyzed (Fig. 5).

addition of hirudin (1 unit per ml) to the incubation mixture of rhTPO (50 μg/ml) and thrombin (5 units per ml) at 37°C (data not shown).

To further classify the domains of thrombin-cleaved peptide fragments, the thrombin-digested samples were probed by six different anti-TPO peptide Abs against HT1, HT2, HT3, HT4, HT5, and HT6 peptide regions (Fig. 3). The results indicated that peptide fragment FA was stained with anti-HT5 and anti-HT6 Abs in samples taken after ≈0.5–64 hr of incubation, and was also reactive with anti-HT4 Ab after 64 hr. This suggests that the FA consisted mainly of amino acid residues located in the C-terminal domain including HT5 and HT6 regions. On the other hand, FB fragment was positively stained with anti-HT1, anti HT2, anti-HT3, and anti-HT4 Abs in samples taken from 0.5 to 64 hr of incubation, suggesting that FB consisted of amino acid residues from around the N-terminal end to A^{190}. Therefore, thrombin cleaved rhTPO into two fragment species of FA and FB, indicating that a thrombin cleavage site was in a peptide region between HT4 and HT5. FC was positively stained with anti-HT4 Ab, but was unreactive with the others. Accordingly, the FC might contain a peptide region between HT3 and HT4. The FD was positive to both anti-HT1 and anti-HT2 Abs at the incubation periods of 4–64 hr, and was also stained strongly by anti-HT3 Ab over all incubation periods. These results mean that the low M_r peptide fragments were derived mainly from the N-terminal region of rhTPO. The appearance of FD corresponded to the reduction in the amount of FA and FB. Additionally, a peptide fragment consisting of FA and FC that was reactive with anti-HT4 was detected only after 64 hr of incubation, suggesting that FD was generated mainly from FB. Although there were several minor cleavage components [e.g., anti-HT6 positive FD after 8.0 hr of incubation (Fig. 3*F*) and anti-HT4 positive protein between intact rhTPO and FA at 64 hr of incubation (Fig. 3*D*)], the transition of various species of peptide fragments generated by thrombin indicated that the thrombin cleavage first occurred selectively at around A^{190} in the C-terminal region of intact rhTPO generating FA and FB, and the latter was then cleaved to form FC and FD.

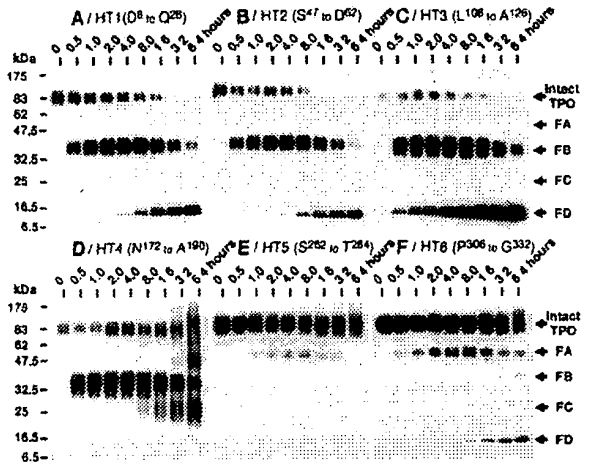

FIG. 3. The transition of domains in thrombin-cleaved components derived from intact rhTPO. Aliquots of thrombin-digested rhTPO, obtained at the same time intervals as described in the legend to Fig. 2, were further analyzed on immunoblots probed with various anti-TPO peptide Abs. Since the sensitivity to the antigen varied among the anti-TPO peptide Abs, appropriate amounts of the aliquots were applied onto SDS/PAGE gels to achieve maximal detection of each antigen. After incubation at 37°C for the times indicated, TPO peptide fragments on each immunoblot were visualized by the ECL method. These Western blots were proved by anti-HT1 Ab, 1 ng of rhTPO per lane (*A*); anti-HT2 Ab, 5 ng of rhTPO per lane (*B*); anti HT3 Ab, 100 ng of rhTPO per lane (*C*); anti-HT4 Ab, 50 ng of rhTPO per lane (*D*); anti-HT5 Ab, 500 ng of rhTPO per lane (*E*); anti-HT6 Ab, 500 ng of rhTPO per lane (*F*). The peptide domains recognized by the anti-TPO peptide Abs here are shown in Fig. 5.

In addition to the analysis of peptide domains, the FDCP-hMpl5 cell proliferation assay revealed that the *in vitro* TPO activity was changed by thrombin digestion as shown in Fig. 4. The total activity of each batch obtained from the time course experiments, a mixture of thrombin-cleaved TPO-peptides, was gradually increased as FB was generated, and reached the maximal activity after 1–4 hr of incubation. The activity then decreased to the basal level in a time-dependent manner, as low M_r products (FC and FD) appeared.

Identification of Thrombin Cleavage Sites. The thrombin sites in proteins were reported by Chang (14). As shown in Fig. 5, we predicted six thrombin sites in human TPO, and they were designated as "Tbn" sites. To identify the actual thrombin cleavage sites, we subjected each major thrombin-cleaved peptide fragment to N-terminal amino acid sequencing together with amino acid composition analysis for the determination of the C-terminal end. As shown in Fig. 5, the FA, FB, FC, and FD fragments were revealed to be peptides consisting of amino acid residues from T^{192} to G^{332}, S^1 to R^{191}, T^{118} to R^{191}, and S^1 to R^{117}, respectively; whereas intact rhTPO was confirmed to be S^1 to G^{332}. In other words, the actual major thrombin cleavage sites in rhTPO were identified as GR^{117}–T^{118} and AR^{191}–T^{192}. Therefore, all predicted thrombin sites were not cleaved under our experimental conditions, though there may be other minor thrombin cleavage sites under different conditions (e.g., denaturing conditions or not, digestion time, pH, concentrations of thrombin and rhTPO, the degree of autolysis of thrombin during digestion, etc.).

DISCUSSION

We found that rhTPO is cleaved in human platelet suspensions containing 1 mM Ca^{2+} (Fig. 1*A*). Because the cleavage of rhTPO in the platelet suspensions was inhibited by hirudin, a thrombin-specific inhibitor, thrombin generated *in situ* seems to be responsible for the cleavage of rhTPO (Fig. 1*B*).

However, thrombin is known to activate other proteases (e.g., protein C). Therefore, naturally we turned to a platelet- and plasma-free system to further investigate the thrombin-induced cleavage of rhTPO. In this study, the major selective thrombin cleavage sites were determined as AR^{191}–T^{192} in the C-terminal domain and GR^{117}–T^{118} in the N-terminal domain of TPO. The sequencing analysis revealed that FC fragment contained the C-terminal region of HT3, whereas FC was not

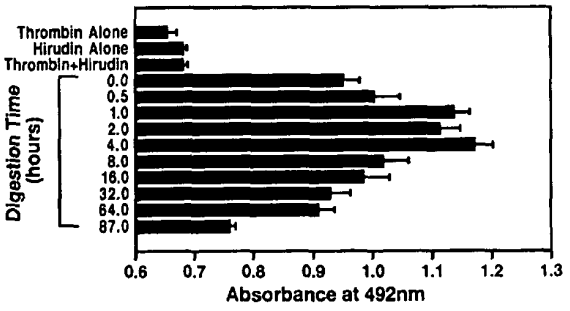

FIG. 4. *In vitro* TPO activity after thrombin digestion. RhTPO (50 μg/ml) was incubated at 37°C in the presence of thrombin (5 units per ml). The reaction was terminated at 0, 0.5, 1.0, 2.0, 4.0, 8.0, 16, 32, 64, or 87 hr by the addition of hirudin (1 unit per ml). Without the addition of rhTPO, the results at 0-hr incubation of thrombin alone, hirudin alone, and thrombin plus hirudin were indicated as negative control. The samples were subjected to the FDCP-hMpl5 cell proliferation assay as described. The data (absorbance at 492 nm) represent the mean ± SD from triplicate assays.

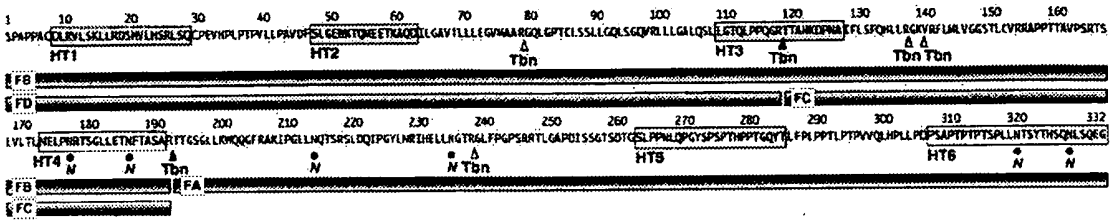

FIG. 5. Antigenic peptide domains recognized by anti-TPO peptide Abs and thrombin cleavage sites of rhTPO. The selected antigenic peptide domains of HT1 (D^{8} to Q^{28}), HT2 (S^{47} to D^{62}), HT3 (L^{108} to A^{126}), HT4 (N^{172} to A^{190}), HT5 (S^{262} to T^{284}) and HT6 (P^{306} to G^{332}) are denoted by shaded boxes. Each amino acid residue in the sequence is shown in the one-letter amino acid code. *N*-glycosylation sites are indicated by a closed circle with an *N*. Thrombin cleavage sites (14) are indicated along a TPO amino acid sequence. In human TPO, there is no site of [P4]-[P3]-[Pro]-[Arg or Lys]-[P1']-[P2'], where P3 and P4 are hydrophobic amino acids and P1' and P2' are non-acidic amino acids. On the other hand, the predicted thrombin sites, which are [P2]-[Arg or Lys]-[P1'], where P2 or P1' is Gly and the [Arg or Lys]-[P1'] bond is cleaved, were found in human TPO at AR^{78}–G^{79}, LR^{136}–G^{137}, GK^{138}–V^{139}, and TR^{237}–G^{238}. In addition, the AR^{191}–T^{192} is one of the other predicted thrombin cleavage sites containing [Ala]-[Arg]-[P5], where the [Arg]-[P5] bond is cleaved, as reported to occur in fibrinogen, chymotrypsinogen A, and antibody λ chain (14). These predicted sites were designated as "Tbn" sites, indicated by triangles. Among them, N-terminal amino acid sequence analyses revealed actual thrombin cleavage sites to be at GR^{117}–T^{118} and AR^{191}–T^{192}, both indicated by closed triangles. The N-terminal amino acid sequences obtained from the major thrombin-cleaved peptide fragments (FA, FB, FC, and FD) and intact rhTPO were as follows: T^{192}TGSGLLKWQQGFRAKIPGL, X(or S^{1}) PAPPAX(or C)DLRVLSKLLRDSH, T^{118}TAHKDPNAIFLSFQHLLRGK, X(or S^{1}) PAPPAX(or C)DLRVLSKLLRDSH, and X(or S^{1}) PAPPAX(or C)DLRVLSKLLRDSH, respectively. Additionally, amino acid composition analysis was conducted to determine each C-terminal end. All results indicated that the major peptide fragments (FA, FB, FC, and FD) consisted of T^{192} to G^{332}, S^{1} to R^{191}, T^{118} to R^{191}, and S^{1} to R^{117}, respectively, as indicated by the open boxes. Both thrombin sites found in human TPO (2, 15, 16) (GR^{117}–T^{118} and AR^{191}–T^{192}) are conserved among TPOs, including mice (16, 18) (GR^{117}–T^{118} and AR^{191}–T^{192}), rats (17) (GR^{117}–T^{118} and AR^{191}–T^{192}), and dogs (16) (GR^{115}–T^{116} and AR^{189}–T^{190}).

strongly recognized by anti-HT3 Ab. It is possible that affinity-purified anti-HT3 Ab may be more reactive against the N-terminal region of the antigen, since it is structurally freer than the C-terminal end of the quadruple-stranded MAP linked together by Lys residues. The analysis of thrombin cleavage analyses by use of anti-TPO peptide Abs revealed that thrombin cleaved rhTPO at selective sites in a discriminated order. Interestingly, thrombin selectively cleaved at AR^{191}–T^{192} of rhTPO first, but did not cleave simultaneously at another site, GR^{117}–T^{118}. We cannot conclude at present whether the removal of the C-terminal polypeptide by cleaving at AR^{191}–T^{192} is structurally required prior to the cleavage at GR^{117}–T^{118}. The result of a test using a truncated TPO rather than a full-length TPO as the initial substrate of thrombin indicated that AR^{191}–T^{192} was hard to be cleaved under nondenaturing conditions (data not shown). It has been predicted that the N-terminal half domain of TPO has a bundle structure consisting of four α-helices with a hydrophobic core based on the analogy with the structure of erythropoietin (29). This may be one of the reasons that the GR^{117}–T^{118} thrombin site in the N-terminal domain of TPO was more resistant against proteolytic cleavage than the AR^{191}–T^{192} site. Since platelets have receptors for thrombin as well as for TPO, it is possible that the association with platelets may affect the structure of rhTPO, and such conformational change of the protein may lead the GR^{117}–T^{118} site to be cleaved easier by thrombin *in vivo*. Taken together, as summarized in Fig. 6, a TPO[1–191] (consisting of amino acid residues from S^{1} at the N-terminal end to R^{191}) was formed first through the cleavage at AR^{191}–T^{192}, and thrombin subsequently cleaved it at the GR^{117}–T^{118}. Moreover, the biological activity of rhTPO was modulated by thrombin cleavage (Fig. 4), though the activity of each thrombin-generated peptide was not individually examined in this study. The *in vitro* TPO activity was increased as FB was generated, indicating that the generation of TPO[1–191] (30) raised the *in vitro* activity. On the contrary, the further thrombin cleavage at GR^{117}–T^{118} reduced or destroyed it. Two disulfide bonds of C^{7}–C^{151} and C^{29}–C^{85} have been identified in rhTPO, and both disulfide loops are essential for exhibiting the TPO activity (30). It should be noted that the peptide fragments of FD (S^{1} to R^{117}) and FC (T^{118} to R^{191}) were still connected to each other via disulfide bonds in the biological assay, whereas they migrated separately on SDS/PAGE gels under reducing conditions. Therefore, further analysis should be conducted to comprehend better how thrombin cleavage affects the biological activity of TPO.

To date, the biochemical information of the native plasma TPO is not sufficient to allow complete understanding about the proteolytic processing that generates truncated TPOs. The molecular masses estimated by SDS/PAGE of porcine (2), ovine (3), rat (4), and canine (5) plasma TPOs was reported to be 28–32 kDa and 18–20 kDa, 31.2 kDa and 36.7 kDa, 19 kDa, and 25 kDa and 31 kDa, respectively. Thrombin cleavage sites exist in rat, murine, and canine TPOs as well as in human TPO. However, the proteolysis by thrombin cannot account for the generation of all the reported purified TPO species in the light of their M_r. Some mechanisms other than the thrombin cleavage may be required for the generation of truncated species around 20 kDa. This supports the notion that other specific proteolytic enzymes or mechanisms may exist and process TPO protein (1, 4, 7).

The most vital point in this study is that thrombin derived from a washed platelet preparation is capable of cleaving TPO. The finding in this study must be taken into account in future studies to examine the "metabolism" of TPO by platelets. Recently, we have reported that the circulating TPO in human

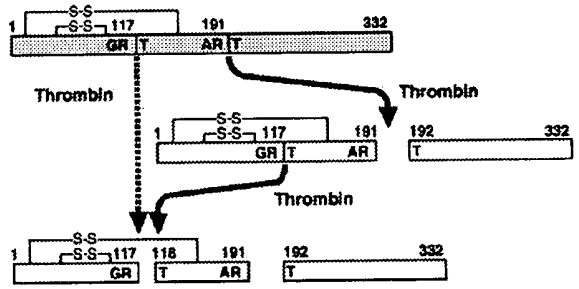

FIG. 6. Schematic thrombin cleavage of rhTPO. From the results of the time course analysis of the major thrombin-cleavage peptide fragments, rhTPO was cleaved at AR^{191}–T^{192} first, and TPO[1–191] was generated. Subsequently, the generated TPO[1–191] was also cleaved by prolonged thrombin digestion at GR^{117}–T^{118} in the N-terminal domain. The biological activity of the resultant proteins are consequently modulated by the thrombin cleavage. Thrombin may directly cleave rhTPO at GR^{117}–T^{118} without the cleavage at AR^{191}–T^{192}, as indicated by the dotted arrow; however, structurally this site is more resistant against thrombin cleavage than the AR^{191}–T^{192} site. Disulfide bonds (C^{7}–C^{151} and C^{29}–C^{85}) are indicated by S–S loops.

plasma, which was not purified, was mainly 80 kDa (31). This finding suggests that TPO undergoes proteolysis under some specific conditions. As previously reported (20, 21), it is possible that thrombin locally modulates the activity of TPO and thus plays a role in megakaryopoiesis.

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3:45 pm-5:15 pm Simultaneous Session

CHROMOSOMAL REARRANGEMENTS

Room 2, San Diego Convention Center

Diane C. Arthur and Carol A. Westbrook

3:45 **Foetal origins of the TEL-AML fusion gene. Abstract #2475**
AM Ford, CA Bennett, CM Price, MCA Bruin, ER van Wering and MF Greaves. London, United Kingdom and The Hague, The Netherlands.

4:00 **ELL interacts with two closely related members of a novel gene family, EAF1 and EAF2. Abstract #2476**
F Simone and MJ Thirman. Chicago, IL.

4:15 **Biochemical analyses of inv(16) fusion gene *CBFB-MYH11*. Abstract #2477**
N Adya and PP Liu. Bethesda, MD.

4:30 **Evidence that HRX leukemic fusion proteins form a heterocomplex with the leukemia-associated protein set and protein phosphatase 2A. Abstract #2478**
HT Adler, FS Nallaseth, G Walter and DC Tkachuk. Seattle, WA; San Diego and La Jolla, CA; Seattle, WA.

4:45 **Non-random chromosomal rearrangement, at 4p13, of the RhoH/TTF gene, encoding a small GTPase restrictively expressed in hematopoietic tissues, in non-Hodgkin's lymphoma and multiple myeloma. Abstract #2479**
C Roumier, C Preudhomme, E Dallery-Prudhomme, MP Hildebrand, JL Laï, P Fenaux, JP Kerckaert and S Galiègue-Zouitina. Lille, France.

5:00 **Use of a 10,000 gene cDNA microarray to analyze gene expression patterns in hematological disorders. Abstract #2480**
DT Ross, M Eisen, D Lashkari, G Shuler, M Boguski, J Hudson, D Botstein, D Shalon and PO Brown. Stanford and Fremont, CA; Bethesda, MD and Huntsville, AL.

3:45 pm-5:15 pm Simultaneous Sessi n

MOLECULAR BIOLOGY OF LEUKEMIA

Room 3, San Diego Convention Center

James Downing and Robert E. Gallagher

3:45 **Expression of MLL-AF4 fusi n transcripts in normal and leukemic human hematopoiesis. Abstract #2481**
FM Uckun, ML Crotty, MG Sensel, HN Sather, L Tuel-Ahlgren, NA Heerema, MB Sarquis, BC Bostrom, JB Nachman, PG Steinherz, PS Gaynon and K Herman-Hatten. St Paul, MN and Arcadia, CA.

4:00 **Screening for partial tandem duplication within the MLL gene in patients with acute myeloid leukemia. Abstract #2482**
S Schnittger, U Kinkelin, C Schoch, D Haase, T Haferlach, H Löffler, B Wörmann, W Hiddemann and F Griesinger. Göttingen, Germany.

4:15 **Telomerase activity and telomere length in healthy donors and patients with leukemia, pre- and post-*ex vivo* expansion. Abstract #2483**
M Engelhardt, RT Silver, P Drullisky and MAS Moore. Freiburg, Germany and New York, NY.

4:30 **The anti-apoptotic genes Bcl-X_L and Bcl-2 are overexpressed in quiescent leukemic progenitor cells. Abstract #2484**
M Konopleva, S Zhao, S Jiang, V Snell, X Zhang, JC Reed and M Andreeff. Houston, TX and La Jolla, CA.

4:45 **Isolation and characterisation of a candidate tumour suppressor gene on 6q21 implicated in ALL and NHL. Abstract #2485**
A Jackson, P Panayiotidis and L Foroni. London, United Kingdom.

5:00 **Identification of candidate tumour suppressor gene cDNAs from the chromosomal region 13q14 deleted in B cell malignancy. Abstract #2486**
P Panayiotidis, C Rowntree, K Ganeshaguru, AV Hoffbrand and L Foroni. London, United Kingdom.

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A Single Injection of Pegylated Murine Megakaryocyte Growth and Development Factor (MGDF) Into Mice Is Sufficient to Produce a Profound Stimulation of Megakaryocyte Frequency, Size, and Ploidization

By Julie T. Arnold, Najat C. Daw, Paula E. Stenberg, Deepthi Jayawardene, Deo Kumar Srivastava, and Carl W. Jackson

Despite numerous studies investigating the action of c-*mpl* ligand, no reports have defined the in vivo changes in megakaryocytopoiesis in response to a single injection of this cytokine. Here we compare the kinetics of the megakaryocytopoietic response in C57Bl/6J mice administered 25 μg/kg or 250 μg/kg of pegylated (PEG) murine megakaryocyte growth and development factor (MGDF) as a single intravenous injection. Megakaryocytes of mice treated with MGDF had normal ultrastructure, showing a typical distribution of the demarcation membrane system, α-granules, and other cytoplasmic organelles. Megakaryocyte ploidy, size, and frequency were markedly increased with both MGDF doses. Megakaryocyte ploidy was maximally increased from a modal value of 16N to 64N on day 3, with both doses of MGDF. Similarly, a comparable increase in megakaryocyte size occurred in the two MGDF groups. Increased megakaryocyte size was coupled to the increase in megakaryocyte ploidy, and no evidence for independent regulation of megakaryocyte size within individual ploidy classes was apparent. In contrast to megakaryocyte ploidy and size, the increase in megakaryocyte frequency was markedly different with the two doses of MGDF. The proportion of 2N and 4N cells was increased from a baseline of 0.035% to 0.430% by day 4 in mice treated with the higher dose of MGDF, but only to 0.175% in mice administered 25 μg/kg of MGDF. The marked increase in the pool of these immature megakaryocytes translated to a sustained elevation in the frequency of polyploid megakaryocytes (8N cells and greater). In contrast to the sustained increase in the frequency of polyploid cells, the level of polyploidization was downregulated on days 6 to 10, but normalized by day 14. We conclude that a single injection of MGDF is able to expand the megakaryocytic pool in a dose-dependent manner, which, with subsequent maturation, should lead to an increased rate of platelet production.

THE RECENT purification and cloning of the c-*mpl* ligand[1] or thrombopoietin (TPO),[2-4] and the production of recombinant forms such as megakaryocyte growth and development factor (MGDF),[5] have provided the opportunity to unambiguously define the regulatory effects of this cytokine on megakaryocytopoiesis. Reports on this cytokine, have described its ability to cause a profound increase in circulating platelet levels in mice,[1,2,6] rats,[4] and nonhuman primates,[7,8] presumably as a result of expansion of the megakaryocyte progenitor population[7,9-12] and enhanced megakaryocyte maturation.[4,8,10,12]

In vitro studies have shown that c-*mpl* ligand is capable of inducing megakaryocyte colony formation and augmenting megakaryocyte maturation. c-*mpl* ligand has been shown to specifically induce colony-forming unit-megakaryocyte (CFU-Meg) formation from unfractionated murine bone marrow (BM) cells,[10,13] murine fetal liver cells,[14] and from human peripheral blood (PB) or BM $CD34^+$ cells.[11,12,15-17] Addition of c-*mpl* ligand to human megakaryocytes in culture can increase platelet-specific antigens,[1,5] increase megakaryocyte polyploidy,[12,15] and allow immature megakaryocytes to develop into morphologically normal, mature megakaryocytes capable of forming proplatelet processes.[18] However, the actual process of proplatelet formation appears to be inhibited by the presence of c-*mpl* ligand in the culture medium.[19]

In vivo, daily administration of c-*mpl* ligand to mice caused an increase in de novo platelet synthesis, as determined by ^{35}S incorporation into platelets,[1] and resulted in the circulating platelet count being increased to three to four times the baseline values.[2,20] In nonhuman primates, daily administration of MGDF raised the platelet count threefold within 5 to 7 days[7,8] as a result of increases in megakaryocyte progenitors,[7] megakaryocyte numbers, volume, and ploidy.[8]

In various models of myelosuppression, daily administration of MGDF or thrombopoietin is able to enhance recovery from thrombocytopenia[6,20] and reduce mortality.[6] The ability of thrombopoietin to enhance platelet recovery in myeloablative models has varied. Some studies report an accelerated recovery of platelet numbers in animals administered thrombopoietin after BM or PB progenitor cell transplantation,[21,22] whereas others have not observed this effect.[23,24] If, however, lethally irradiated recipients were transplanted with marrow collected from animals pretreated with thrombopoietin, the thrombocytopenia in the transplanted animals was less marked and shorter in duration.[23] Similarly, irradiated mice receiving PB progenitor cells (PBPC) mobilized by pegylated MGDF[22] were thrombocytopenic for fewer days, compared with recipients of unmobilized PBPC.

In all but one study,[20] MGDF or thrombopoietin has been administered on a daily injection schedule, ranging in duration from 2 to 10 days,[2,4,6,7,20] to 4 weeks.[8] The choice of a daily injection schedule has been based on the use of other hematopoietic cytokines[25-27] rather than the pharmacokinet-

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ics of thrombopoietin. More importantly, no detailed analysis of the in vivo kinetics of megakaryocytopoiesis in response to a single administrative dose has been reported. To address this issue and with the rationale that an understanding of megakaryocyte kinetics would aid the design of a clinically effective administration schedule, we have defined the megakaryocytic response in C57Bl/6J mice administered a single intravenous injection of pegylated recombinant murine MGDF (PEG-rmMGDF).

MATERIALS AND METHODS

Young adult C57Bl/6J male mice were purchased from The Jackson Laboratories (Bar Harbor, ME) and ranged between 10 and 20 weeks of age at the time of study.

PEG-rmMGDF was kindly supplied by Amgen Inc (Thousand Oaks, CA). The stock solution of PEG-rmMGDF (usually 0.63 mg/mL) was diluted with 1% normal (homologous) mouse serum (NMS) in phosphate-buffered saline (PBS) on the day of administration. A standard 2.5 μg/mL and 25 μg/mL solution of rmMGDF was made for injection at the 25-μg/kg and 250-μg/kg doses, respectively. Each mouse received 0.1 mL per 10 g body weight, whereas control mice received an equivalent volume of 1% NMS. MGDF or carrier were administered intravenously via the lateral tail vein on day 0, after inducing vasodilation by warming the mice for 10 to 20 minutes under an examination lamp.

Flow cytometric analysis of megakaryocytes. Mice were anesthetized with methoxyflurane and killed by exsanguination at frequent intervals from 12 hours to 14 days after MGDF administration. Marrow from one femur and one tibia was flushed into 2 mL of CATCH medium[28] containing 8 μmol/L prostaglandin-E_1 (PGE_1) (Sigma, St Louis, MO). Marrow cells were gently resuspended with a plastic, standard-bulb transfer pipette (Fisher, St Louis, MO) and kept on ice. Marrow suspensions were filtered through 105-μm monofilament nylon mesh (Small Parts Inc, Miami, FL) to remove cell clumps and debris. The cells were pelleted at 400g for 5 minutes at room temperature (RT), and then resuspended in 1 mL of CATCH diluted 1:1 with PBS containing 5% normal goat serum (NGS) (GIBCO-BRL, Life Technologies Inc, Grand Island, NY). NGS was used to block nonspecific binding of goat-derived secondary antibody.

Megakaryocytes were labeled for 60 minutes on ice with a saturating concentration of a rat monoclonal antibody to mouse platelets (4A5), and shown to have a high specificity for murine megakaryocytes[29] (kindly provided by Dr S. Burstein, University of Oklahoma, Oklahoma City). Cells were pelleted at 400g for 5 minutes at RT and resuspended in 1 mL of CATCH diluted 1:1 with PBS containing 5% NGS. The megakaryocyte-bound 4A5 antibody was indirectly fluoresceinated with fluorescein isothiocyanate (FITC)-goat anti-rat IgG $F(ab')_2$ (Biosource, Camarillo, CA) for 30 minutes on ice. Marrow cells labelled with only FITC-goat anti-rat IgG $F(ab')_2$ served as the negative control. In MGDF-treated animals, an increased concentration of 4A5 and FITC-labeled second antibody was added to the samples when megakaryocyte frequency and size were increased to ensure saturation of 4A5 binding sites, as monitored by the level of green fluorescence.

After labeling with primary and secondary antibody, the cells were pelleted by centrifugation at 400g for 5 minutes at RT, and resuspended in 2 mL of hypotonic propidium iodide (50 μg/mL in 0.1% sodium citrate).[30] Before flow cytometric analysis, the cells were treated with RNAase (50 μg/mL, bovine pancreas; Calibiochem Corp, San Diego, CA), and filtered through 105-μm nylon mesh.

DNA content of 4A5-positive cells was analyzed by two-color flow cytometry[31] on a FACScan (Becton-Dickinson, San Jose, CA). DNA contents of all 4A5-positive cells were measured (DNA contents 2N-128N). 4A5 positivity was defined by setting the horizontal (FL-1, green fluorescence) window at a level to minimize 6N events. We have previously shown[31] that most all 6N events are aggregates of other marrow cells and thus serve as an internal negative control for the green window selection. Marrow cells from control animals were always used to set this gate and the same gate used to define 4A5-positive cells in marrow from MGDF-treated animals. This level of green fluorescence was associated specifically with 4A5-binding, as marrow labeled with FITC-second antibody alone showed few if any cells in the defined 4A5-positive window. The proportion of cells in each ploidy class was determined by integrating the number of cells under each DNA peak. Megakaryocyte frequency was calculated from the flow cytometric profiles as the percentage of cells expressing 4A5. Cell size was indexed by forward-angle light-scatter (FSC) and the mean megakaryocyte size of polyploid cells was determined from the two-color profile.

Collection and preparation of BM for transmission electron microscopy. Mice were killed 4 days after administration of 25 μg/kg, 250 μg/kg rmMGDF, or 1% NMS. BM was gently flushed from the tibia of each mouse into 2 mL of fixative composed of 1.5% glutaraldehyde + 1.5% paraformaldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.4. Two-percent dimethyl sulfoxide (DMSO) was added, and samples promptly microwaved for 10 seconds and fixed overnight at 4°C. The marrow was rinsed in 0.1 mol/L sodium cacodylate buffer, pH 7.4, and then postfixed in 2% osmium tetroxide in 0.1 mol/L sodium cacodylate buffer for 1.5 hours. The samples were dehydrated through a series of graded ethanol, infiltrated with toluene and subsequently with resin composed of DDSA, NMA, and DMP-30 (Ted Pella, Redding, CA) and LX-112 (Ladd Research Industries, Burlington, VT), and finally embedded in 100% of the above resin. Thin sections were cut and stained with uranyl acetate and lead citrate and viewed with a Philips 301 transmission electron microscope (Philips Electronic Instruments, Inc, Mahwah, NJ).

Statistical analyses. Statistical comparison of megakaryocyte ploidy distributions is complex and previous studies performed on megakaryocyte ploidy have either neglected to report statistics, compared the proportion of cells in each class using univariate two-sample t[32] or Mann-Whitney rank sum[33-38] test, or used a summary statistic of all ploidy classes, such as the geometric mean,[39] again compared using univariate test procedures.[4,40,41] However, these approaches do not consider that the proportion of cells in each of the DNA classes are interrelated. A more appropriate procedure, and one we have implemented in our studies, is the Hotelling's T^2 test,[42] which is a multivariate test procedure for comparing two population mean vectors and takes into account the underlying covariance structure. This allowed us to analyze megakaryocyte ploidy by comparing the distribution (all DNA classes simultaneously) in the control group and the distribution at each of the time points for each MGDF dosage. Hotelling's T^2 test is appropriate if the underlying distribution function for the two populations can be assumed to be multivariate normal. However, due to the relatively small sample sizes of our data, we did not wish to make this assumption, and therefore used a Permutation test[43] of Hotelling's T^2 to evaluate the differences in the ploidy distributions. The following is an example of how the data were analyzed using the permutation/Hotelling's T^2 test. For comparison of the ploidy distributions for the control group (13 mice) and the group of mice killed 12 hours after the administration of 25 μg/kg of rmMGDF (5 mice), we first obtained the value of Hotelling's T^2 statistic for the original sample and denoted it by T_0^2. The Hotelling's T^2 statistic was then calculated for all possible samples that could be obtained by allocating 13 of the 18 mice into the control group and the remainder to the treatment group. The total number of permutations for this Hotelling's T^2 test was 8,568. The P value was obtained by counting the number of times we observed a value of T^2 greater than T_0^2 and dividing it by the total

number of permutations (8,568). When the total number of permutations was less than 17,000 for a particular comparison, then all the possible permutations were used in obtaining the P value (a permutation test by exact methods). If not, a random sample (Monte Carlo modication) of 17,000 permutations was chosen to obtain the P value. With 17,000 permutations, we were assured that the estimated P value would be within a 1% margin of error of the true P value with 99% confidence.[44] A program was written in MINITAB[45] (MINITAB, Inc, PWS-KENT Publishing Co, Boston, MA) to calculate Hotelling's T^2 and implement the permutation test. Because 13 multiple comparisons were made (comparing ploidy distribution at each time point to the control), a Bonferroni correction[46] was used to maintain the overall experimental type I error level of $\alpha = .05$ (a 95% confidence limit of detecting a real difference when there is one). Therefore, in each comparison the observed P value had to be less than .004 (.05/13) to be judged significant. Type I error is the probability of rejecting the null hypothesis and concluding that there is a significant difference between the two groups when in fact there is none.

The effect of MGDF treatment with time on megakaryocyte frequency (of both 2N/4N and 8N-128N cells) and size in mice treated with 25 μg/kg and 250 μg/kg rmMGDF was analyzed using the Kruskal-Wallis test,[47] a nonparametric test procedure. Once an effect of rmMGDF on a given megakaryocyte parameter was detected, a posthoc analysis comparing the control group to all other (13) time points was done using Wilcoxon-Mann-Whitney rank sum test.[47] Because of the small sample sizes, the permutation test as implemented in the statistical software package STATXACT[48] (CYTEL Software Corp, Cambridge, MA) was used. When the total number of permutations was large, the Monte Carlo estimate of the P value was based on 10,000 permutations. With 10,000 permutations, we can be 99% confident that the estimated P value will be within 1.3% margin of error of the true P value. Once again the Bonferroni correction was used so that the posthoc comparisons were performed at level where the P value had to be less than .004 (.05/13) to be significant.

All results are graphed showing the arithmetic mean ± standard error of the mean (SEM), although most of the statistical procedures used are nonparametric in nature and involve comparison of medians rather than means.

RESULTS

Megakaryocyte ploidy. The DNA ploidy classes were well resolved by the flow cytometric method at all time points after MGDF administration. This is illustrated in Fig 1, where representative DNA content distributions are shown for the two extremes of response (maximal stimulation and downregulation) to MGDF. Mean histograms derived from the results of individual flow cytometric profiles of DNA content are presented in Fig 2.

The modal polyploid DNA class for control C57Bl/6J mouse megakaryocytes was 16N (Fig 2); approximately two thirds of the polyploid megakaryocyte population were 16N, with 10% 8N, and 20% 32N. Less than 3% of cells had a DNA content of 64N or greater (Fig 2). A significantly altered polyploid distribution was observed from 12 hours until day 9 (25 μg/kg) or day 10 (250 μg/kg), following administration of a single injection of MGDF. An increase in megakaryocyte ploidy was detected as early as 12 hours, and the response was evident with both doses of MGDF ($P \leq .002$ for both doses, based on permutation/Hotelling T^2 test) (Fig 2). The combined relative frequency of 64N and

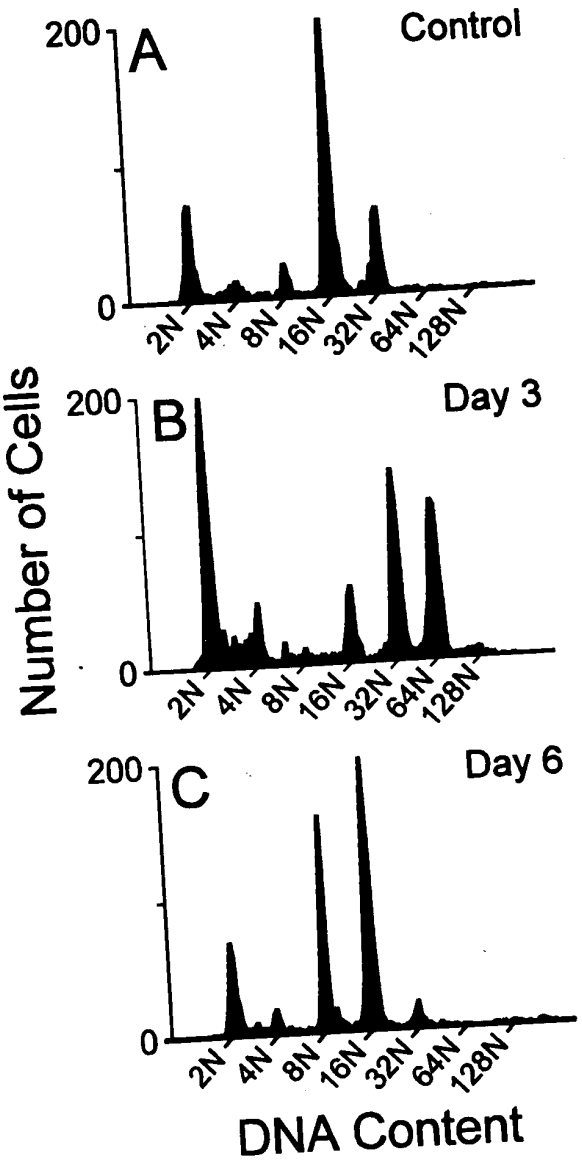

Fig 1. Representative megakaryocyte DNA distributions from the BM of individual mice given 1% mouse serum (A), or treated with 25 μg/kg rmMGDF and killed on day 3 (B) or day 6 (C).

128N cells at 12 hours had increased from 3.0% to 12.0% (both doses), with a corresponding decrease of 16N cells, whereas the 32N frequency remained relatively unchanged. By 24 hours, the modal ploidy class was increased to 32N in mice administered 250 μg/kg of MGDF. 16N remained modal with the lower dose of MGDF; however, the relative frequency of 32N cells was approximately doubled from control values. By 36 hours, the modal megakaryocyte ploidy class had increased to 32N, in mice treated with either dose of MGDF, when the 32N cells represented over 50% of the polyploid population. The 64N and 128N cells comprised 20%, and the 8N and 16N cells less than 30%. The ploidy of the megakaryocytes continued to increase over the next

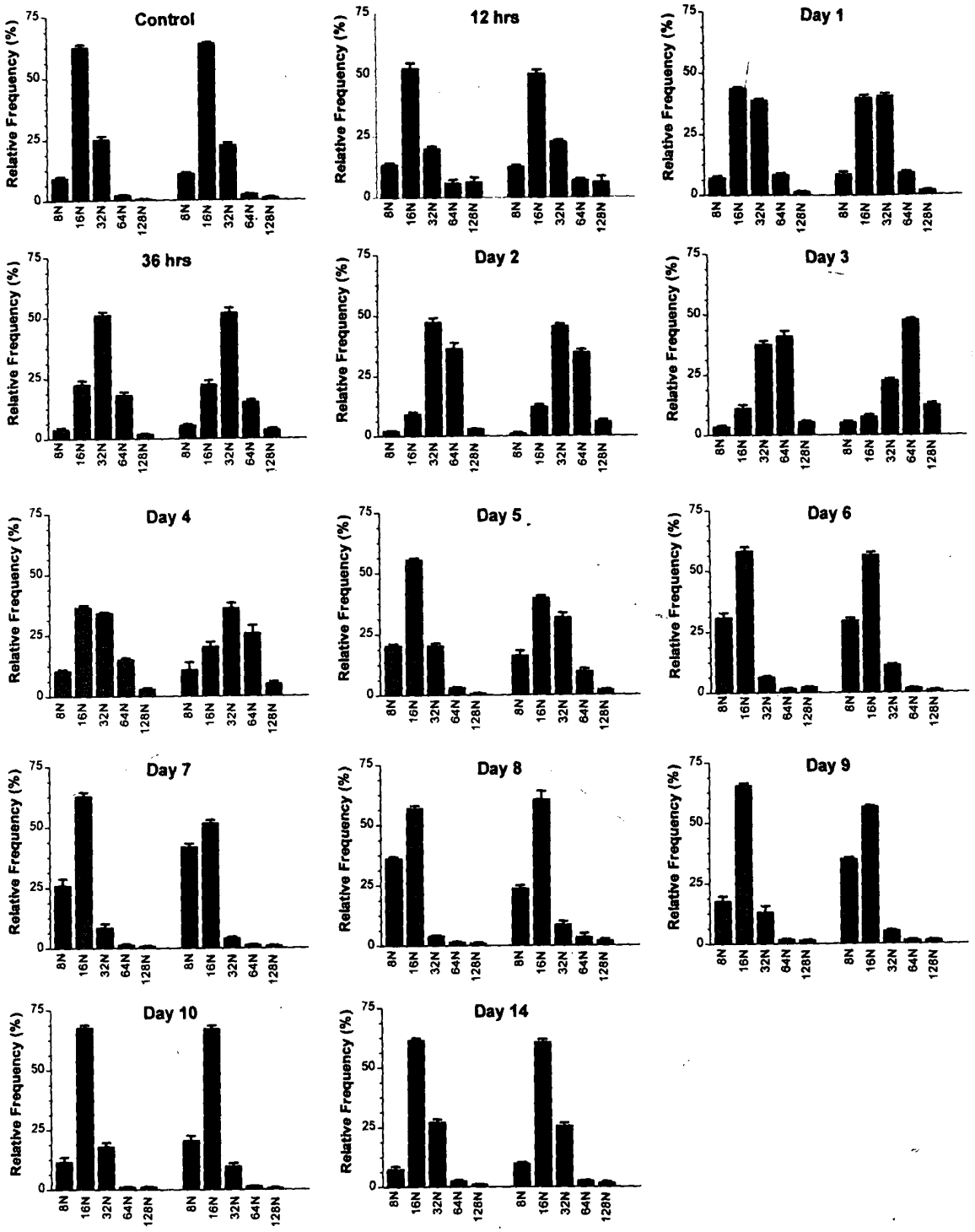

Fig 2. Effects of a single intravenous injection of rmMGDF on megakaryocyte ploidy distributions in mice treated with 25 μg/kg (■) or 250 μg/kg (■) MGDF. Megakaryocyte ploidy distributions were significantly different from the control distribution from 12 hours to day 9 in mice treated with 25 μg/kg of MGDF, and from 12 hours to day 10 in mice treated with 250 μg/kg of MGDF. From 387 to 1,136 (median 871) 4A5 positive cells with DNA contents ≥ 8N were analyzed per mouse. Each distribution shows the mean ± SEM of each megakaryocyte ploidy class; for the 25-μg/kg dose, n = 4-10 mice per MGDF group and n = 13 for controls; for the 250-μg/kg dose, n = 5-9 mice per MGDF group and n = 20 for controls. Controls received 0.1 mL/10 g body weight of 1% NMS in PBS.

36 hours, and peaked at day 3, with 64N as the modal ploidy class at both MGDF doses. The relative frequency of 64N cells was slightly higher for the 250-μg/kg dose (48%) than for the 25-μg/kg dose (41%) and the frequency of 128N cells with the higher MGDF dose was double (13%) that of the lower dose (5%). By day 4, megakaryocyte ploidy began to shift back toward normal, where 32N was the modal ploidy with the 250-μg/kg dose and 16N was the modal for the 25-μg/kg dose. By day 5, the modal ploidy class had returned to 16N in mice treated with either dose of MGDF; however, the ploidy distribution remained significantly different from control ($P < .0001$ for both doses, permutation/Hotelling T^2 test) due to the notable increase in the relative frequency of 8N cells. On day 6, in mice administered 25 μg/kg of MGDF, 8N cells were three times, whereas 32N cells were one fifth, the relative frequency found in control BM ($P < .0001$, permutation/Hotelling T^2 test). On day 7, in mice administered 250 μg/kg of MGDF, 8N cells were four times and 32N cells were one fifth the relative frequency found in control marrow ($P < .0001$, permutation/Hotelling T^2 test). 16N cells in both cases were only marginally below control values. These data suggest that megakaryocyte endomitosis was less stimulated in these mice and the majority of cells were not able to undergo more than three endomitotic cycles. By day 9, ploidy began to normalize in mice treated with 25 μg/kg of MGDF, and by day 10 the relative frequency in each ploidy class was no different from that of megakaryocytes from control BM ($P = .006$, permutation/Hotelling T^2 test). Megakaryocyte ploidy of mice receiving the higher MGDF dose showed a similar pattern, but normalization of the ploidy distribution did not occur until day 14 ($P = .408$, permutation/Hotelling T^2 test).

Megakaryocyte frequency. We chose to separate the megakaryocytes into two populations for frequency analysis: those with a presumed capacity for cell division, cells of 2N/4N DNA content, and those cells that had ceased dividing and were undergoing or had completed polyploidization (8N, 16N, 32N, 64N, and 128N cells). We realize that this division of cells has its limitations and is unable to discriminate the 2N cells not in cell cycle, or the 4N population that is truly polyploid, but it provides an estimate of the proliferating (2N/4N cells) versus the nonproliferating (8N-128N) megakaryocyte population.

Previous morphometric studies have estimated the frequency of morphologic recognizable megakaryocytes in normal BM to be 0.05%.[36] Our current study, using flow cytometric analysis, estimated that the frequency of 4A5-positive polyploid cells (presumably the morphologically recognizable megakaryocytes) to be approximately 0.08%. The combined frequency of 4A5-positive 2N and 4N cells was approximately 0.035%.

Administration of either 25 μg/kg or 250 μg/kg of rmMGDF had a significant effect on the frequency of 2N/4N cells ($P < .0001$, Kruskal-Wallis, both doses) (Fig 3A). The frequency of the 2N/4N cells in mice administered 25 μg/kg of MGDF remained constant for 36 hours after cytokine administration (Fig 3A). At day 2, the frequency of 2N/4N cells started to increase ($P = .001$, Mann-Whitney); by day 4, the 2N/4N cell frequency had peaked at 0.175%, a fivefold increase from baseline values ($P < .0001$, Mann-Whitney). By day 5, 2N/4N frequency promptly returned to baseline values ($P = .13$, Mann-Whitney) and remained not significantly different from controls through to day 14. Mice administered 250 μg/kg of MGDF showed similar kinetics in the 2N/4N frequency. The first increase was again detected on day 2 ($P < .0001$, Mann-Whitney), and the frequency increased to a maximum of 0.430% on day 4 ($P < .0001$, Mann-Whitney), representing a 10-fold increase over control values, or twice the increase observed with the 25-μg/kg dose. 2N/4N frequency sharply decreased to 0.125% on day 5, but in contrast to mice treated with the lower dose of MGDF, 2N/4N frequency was still significantly increased from baseline ($P < .0001$, Mann-Whitney). By day 6, 2N/4N frequency had normalized ($P = .11$, Mann-Whitney), and remained normal through to day 14.

Administration of either 25 μg/kg or 250 μg/kg rmMGDF

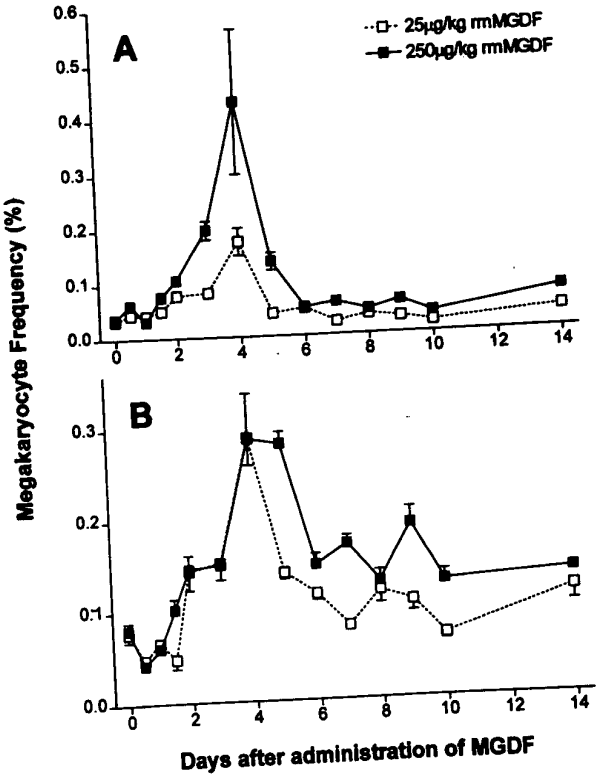

Fig 3. Effect of a single intravenous injection of rmMGDF on the frequency of (A) 2N-4N megakaryocytes and (B) 8N-128N polyploid megakaryocytes. The frequency of 2N/4N cells was significantly increased from days 2 to 4 in mice treated with 25 μg/kg of MGDF and from days 2 to 5 in mice treated with 250 μg/kg ofMGDF (A). The frequency of 8N-128N cells was significantly increased from days 3 to 5 in mice treated with 25 μg/kg MGDF and from days 2 to 14 in mice treated with 250 μg/kg MGDF (B). At 12 hours, the frequency of 8N-128N cells was significantly decreased with the 250-μg/kg dose of MGDF (B). Each data point represents the mean ± SEM of megakaryocyte frequency; for the 25-μg/kg dose, n = 4-10 mice per MGDF group and n = 13 for controls and for the 250-μg/kg dose, n = 5-9 mice per MGDF group and n = 20 for controls. Data from control mice were pooled to represent the day 0 data point.

also had a significant effect on the frequency of polyploid megakaryocytes ($P < .0001$, Kruskal-Wallis, both doses) (Fig 3B). The frequency of polyploid megakaryocytes, 12 hours after administration of either 25 or 250 μg/kg of MGDF, appeared to decrease, but this decrease was only found to be significant with the 250 μg/kg dose ($P = .002$ for 250 μg/kg; $P = .06$ for 25 μg/kg, Mann-Whitney). By day 3, the frequency of polyploid megakaryocytes was twofold of normal in mice treated with 25 μg/kg of MGDF ($P = .003$, Mann-Whitney), and continued to increase, reaching a maximal frequency of 0.289% (fourfold of normal) on day 4 ($P < .0001$, Mann-Whitney). After this time, the frequency of polyploid megakaryocytes decreased, and although still twofold of normal by day 5 ($P = .003$, Mann-Whitney), it was not significantly different from control values from day 6 onward. In contrast, mice treated with 250 μg/kg MGDF had an earlier and sustained increase in the frequency of polyploid megakaryocytes. By day 2, the frequency of polyploid megakaryocytes was increased ($P = .0009$, Mann-Whitney) and continued to rise to a maxima of 0.288% (fourfold of normal) on day 4 ($P < .0001$, Mann-Whitney). On day 5, the frequency of polyploid megakaryocytes in mice treated with 250 μg/kg of MGDF remained at fourfold of control ($P < .0001$, Mann-Whitney), decreased to twofold of normal by day 6 ($P = .002$, Mann-Whitney), and was sustained at a significantly elevated level through to day 14 ($P \leq .003$, Mann-Whitney).

Megakaryocyte size. Flow cytometric measurement of FSC is an index of cell size. In our studies, we analyzed the average FSC of polyploid megakaryocytes to assess the effect of MGDF on megakaryocyte size. The megakaryocytes sized were the same cells analyzed for ploidy and frequency.

Administration of either 25 μg/kg or 250 μg/kg rmMGDF significantly altered mean megakaryocyte size of 8N-128N cells ($P < .0001$, Kruskal-Wallis, both doses) (Fig 4), with similar changes observed in both treatment groups. No change in mean megakaryocyte size was apparent until day 2, when average size increased to approximately 130% of normal with both MGDF doses ($P = .001$, 25 μg/kg; $P = .0004$, 250 μg/kg, Mann-Whitney). Mean megakaryocyte size remained elevated on day 3 ($P = .001$, 25 μg/kg; $P = .003$, 250 μg/kg, Mann-Whitney), but by day 5 megakaryocyte size had returned to control values. Although average sizes tended to be smaller than control values on days 6-7 in mice treated with 25 μg/kg MGDF, and on days 6 through 9 in mice treated with 250 μg/kg MGDF ($P \geq .02$, Mann-Whitney, both doses), these values were associated with a P value $> .004$, which we selected for our significance level.

To determine if the increase in mean megakaryocyte size was related to the increase in megakaryocyte ploidy, megakaryocyte size was measured within each ploidy group (Fig 5). As shown, the average size of megakaryocytes increased, with increasing ploidy (Fig 5A and B). Although mean megakaryocyte size was elevated on day 2 with both doses of MGDF (see inset of Fig 5A and B), there was no change in the size of the megakaryocytes within each ploidy group; rather, the increase in mean megakaryocyte size observed on day 2 is a reflection of the modal ploidy shift from 16N to 32N observed with both doses of MGDF (Fig 2). As the modal ploidy returned to 16N on day 7, mean megakaryocyte size also returned to control values.

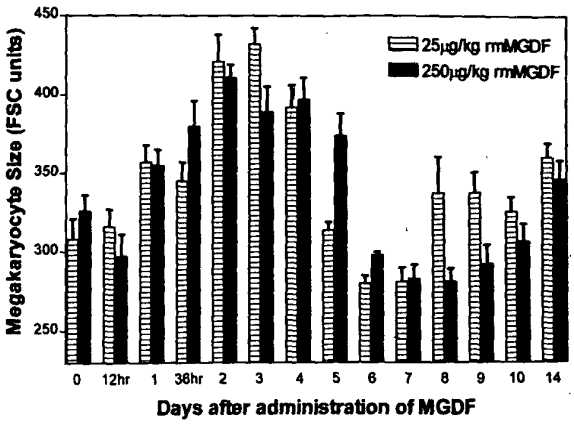

Fig 4. Effect of a single intravenous injection of rmMGDF on average size of polyploid megakaryocytes. The mean megakaryocyte size of 8N-128N cells was significantly increased from days 2 to 4 in mice treated with 25 μg/kg of MGDF, and on days 2 and 3 in mice treated with 250 μg/kg of MGDF. Each data point represents the mean ± SEM of megakaryocyte size (8N-128N cells); for the 25-μg/kg dose, n = 4-10 mice per MGDF group and n = 13 for controls and for the 250-μg/kg dose, n = 5-9 mice per MGDF group and n = 20 for controls. Data from control mice were pooled to represent the day 0 data point.

Megakaryocyte ultrastructure. Megakaryocyte ultrastructure was examined on day 4 (Fig 6), at the peak of the increased frequency of polyploid megakaryocytes (Fig 3B). BM from mice administered 1% NMS (Fig 6a), 25 μg/kg MGDF (not shown), or 250 μg/kg MGDF (Fig 6b) was examined by transmission electron microscopy. The ultrastructure of the megakaryocyte cytoplasm was normal in both groups of MGDF-treated mice. Although the increase in megakaryocyte size of MGDF-treated animals was apparent on day 4 (Fig 6b), the cytoplasmic distribution of the demarcation membrane system (DMS), α-granules, and other organelles was similar to that of the NMS-treated animals (Fig 6a).

DISCUSSION

Preclinical studies[6-8,20] and early data from phase I trials[49-51] clearly indicate that recombinant thrombopoietin will prove useful in the clinic to ameliorate thrombocytopenia, especially that associated with chemotherapy.[6,20,52] In our preliminary experiments[53] we noted that a single intravenous injection of PEG-rmMGDF was just as effective at elevating the platelet count threefold to fivefold of normal, as the multiple injection schedules used in these preclinical studies.[6-8] Therefore, the aim of the present study was to determine the mechanism by which c-*mpl* ligand alters megakaryocytopoiesis to induce an increase in platelet production. To achieve this aim, we analyzed BM frequency of both the megakaryocyte precursor and the polyploid population, megakaryocyte DNA content, and megakaryocyte size at frequent intervals for 2 weeks after a single injection of MGDF to mice.

Our results show that a single injection of PEG-rmMGDF

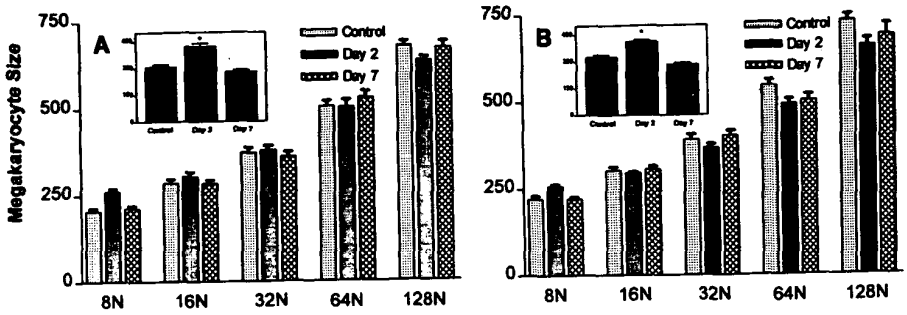

Fig 5. Effect of rmMGDF on megakaryocyte size within individual ploidy classes in mice treated with 25-μg/kg (A) or 250-μg/kg (B) dose. MGDF causes a significant increase in mean megakaryocyte size (see inset) on day 2, with injection of 25 μg/kg ($P = .001$, Mann-Whitney) or 250 μg/kg ($P = .0004$, Mann-Whitney) MGDF, with little change in the size of megakaryocytes within individual ploidy classes. The increase in mean megakaryocyte size results from an increase in the relative frequency of large polyploid cells. On day 2, 32N is the modal DNA class, while by day 7 16N is once again the normal modal class. Each data point represents the mean ± SEM of megakaryocyte size. For the 25-μg/kg dose, n = 13 for the control and n = 5 on days 2 and 7. For the 250-μg/kg dose, n = 20 for the control, and n = 5 on day 2 and n = 8 on day 7.

(25 μg/kg or 250 μg/kg) is able to substantially elevate megakaryocyte frequency and cause a marked shift in modal DNA content, which is accompanied by an increase in mean megakaryocyte size. The implication of these findings is that MGDF is capable of influencing both megakaryocytic proliferation and differentiation, a property that the classically defined thrombopoietin was thought to lack.[54,55]

Our data clearly indicate that MGDF acts on a proliferative megakaryocytic 2N precursor, and the degree of expansion of this compartment is dose dependent. Although 25 μg/kg of MGDF was able to expand the 2N/4N population fivefold, the higher dose of MGDF increased this population to 10-fold of normal. The greater expansion observed with 250 μg/kg of MGDF translated to a sustained increase in the frequency of polyploid megakaryocytes from days 3 to 14. In contrast, increased frequency of polyploid megakaryocytes was of only 3 days duration with the 25 μg/kg dose (days 3-5).

The exact nature of the 2N precursor population stimulated by MGDF in our studies is unknown. The antigen to which the 4A5 antibody is directed has been characterized as a thrombin-sensitive 74-kD glycoprotein present on murine megakaryocytes and platelets.[56] Whether this antibody is capable of binding and labeling the CFU-Meg, or is restricted to only the transitional small acetylcholinesterase positive ($SACHE^+$) cells,[57] has not been determined. Our studies indicate that 48 hours were required to see an increase in the frequency of the 2N population, and the timing was independent of the dose given. However, the 2N population must have a short transition to the endomitotic compartment, as the increase in frequency of polyploid cells was observed only 24 hours later, on day 3. Therefore, the data suggest that a relatively late megakaryocyte progenitor (CFU-Meg) and the population of $SACHE^+$ cells capable of cell division[57] are a target for MGDF. Similar conclusions were reached from in vitro studies using isolated human marrow progenitors,[15,58] showing that a subpopulation of megakaryocyte progenitors ($CD34^+$, $CD41^+$ cells) expressed the receptor, c-*mpl*,[58] and may be the main target cells for thrombopoietin.[15] These cells were shown to have limited capacity for cell division, usually generating colonies of small numbers of megakaryocytes.[15] Furthermore, mice genetically engineered to be deficient in the thrombopoietin receptor (c-$mpl^{-/-}$), showed a greater deficiency in the more mature committed CFU-Meg of limited proliferative potential than in less mature megakaryocyte progenitors.[59] All these data suggest that relatively late megakaryocyte precursors are acutely responsive to changes in thrombopoietin levels and may play a pivotal role in accelerating megakaryocytopoiesis when there is an increased platelet demand.

A second major effect of MGDF is the profound stimulation of megakaryocyte endomitosis. The kinetics, although perhaps not the magnitude of stimulation, were similar to that observed in animals made acutely thrombocytopenic.[60,61] As early as 12 hours after MGDF administration, a marked increase in the proportion of 64N and 128N cells was seen. Interestingly, the relative frequency of 32N cells, remained essentially unchanged. The increase in frequency of 64N and 128N cells must have resulted from stimulation of lower ploidy megakaryocytes (8N, 16N, 32N) to undergo a least one to two extra rounds of endomitosis within 12 hours. Given the steady-state cell-cycle time for rodent megakaryocytes of 10 hours,[62] the data suggest that MGDF was able to shorten the cell cycle time for endomitotic megakaryocytes. An accelerated rate of megakaryocyte maturation has been reported in animals recovering from acute thrombocytopenia, where endogenous levels of thrombopoietin would be expected to be high.[63] The maximal shift in megakaryocyte ploidy was observed at 3 days, where 64N was the modal polyploid class and nearly 50% of all megakaryocytes had a ploidy value of 64N or greater, compared with only 3% in control marrow.

Accompanying the increase in megakaryocyte ploidy was an increase in mean megakaryocyte size. There was no evidence of any increase in megakaryocyte size within a specific ploidy class. Rather, the increase in frequency of high ploidy megakaryocytes (which were always larger in size) produced the increase in mean megakaryocyte size. Mean megakaryocyte size was increased on days 2 and 3, when the modal megakaryocyte ploidy was either 32N or 64N. By day 5, when the modal ploidy had returned to the

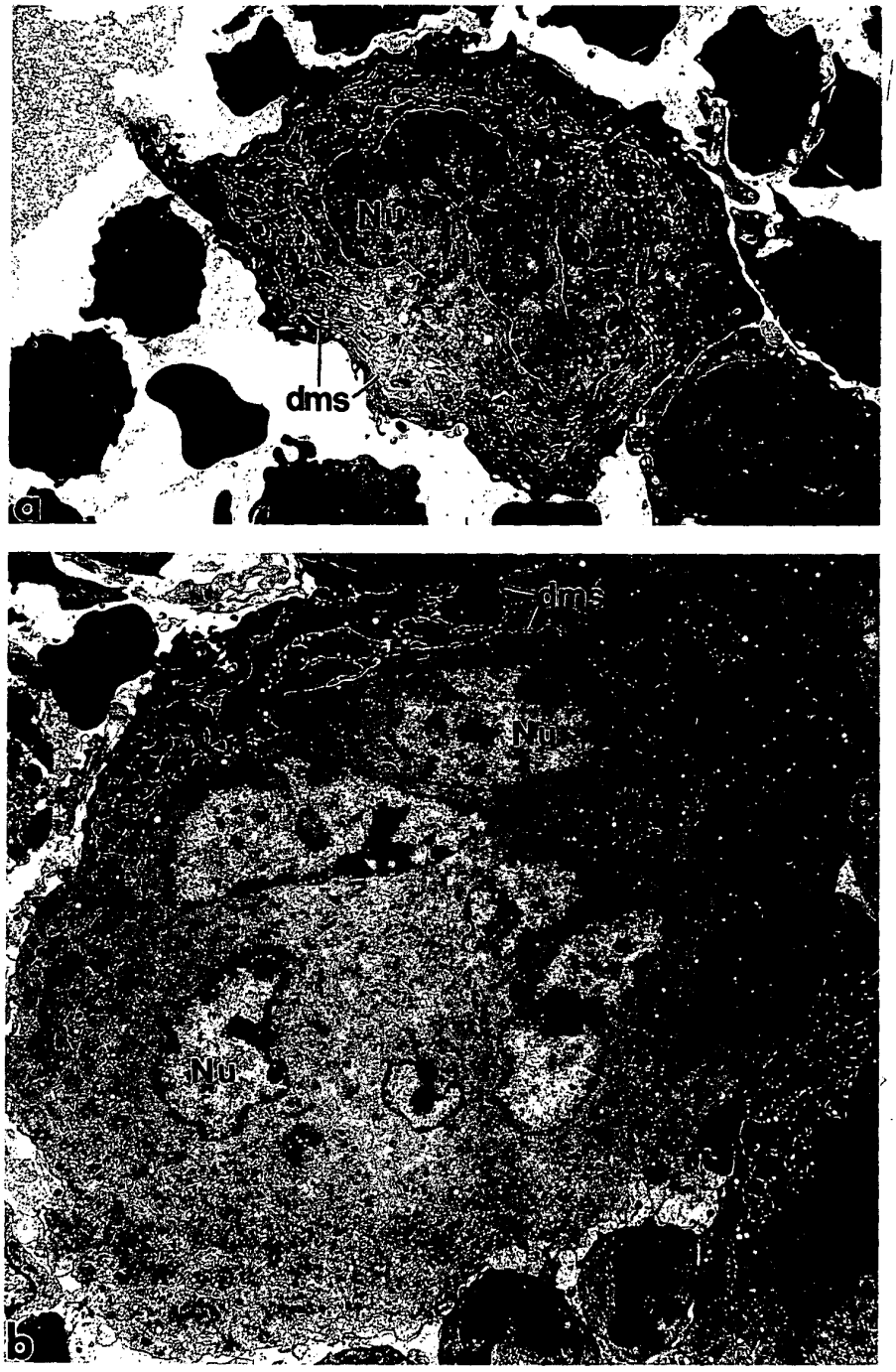

Fig 6. Transmission electron micrographs of BM megakaryocytes from a mouse injected with 1% NMS (a) and a mouse injected with 250 μg/kg rmMGDF (b). Although considerably larger in size than cells from control animals, the megakaryocytes from MGDF-treated animals contain normally distributed organelles within their cytoplasm, including the demarcation membrane system. Nu, nucleus; dms, demarcation membrane system. Original magnification × 4,000.

normal value of 16N, megakaryocyte size also returned to control values.

Our electron microscopic analysis of megakaryocyte morphology showed that MGDF promoted the development of ultrastructurally normal and mature megakaryocytes. We did not observe the maturational defects reported for megakaryocytes generated in culture with thrombopoietin.[15] The most likely explanation for this aberrant megakaryocyte morphology in vitro is an insufficient microenvironment. Variation in exposure and clearance of MGDF may also contribute to the morphologic differences in megakaryocytes observed in vitro versus in vivo.

It has long been hypothesized that the number of platelets in circulation exercise feedback control on megakaryocytopoiesis, including regulation of megakaryocyte DNA content and size.[60,61,64-66] Our data show that polyploidization was

reduced between days 6 and 9 with 25 μg/kg of MGDF and days 6 and 10 with 250 μg/kg of MGDF. In studies reported elsewhere, we showed that a single injection of 25 μg/kg or 250 μg/kg MGDF raised the platelet count to threefold or fivefold on day 5.[53] Therefore, the longer duration of lowered ploidy in mice treated with 250 μg/kg of MGDF was associated with the more pronounced thrombocytosis, and could be interpreted as the role of platelets in this regulatory process. However, at the same time, megakaryocyte number was also higher in mice treated with 250 μg/kg MGDF and increased megakaryocyte frequency may alternatively downregulate polyploidy formation.

A mechanism whereby circulating platelets may affect feedback regulation of megakaryocytopoiesis recently has been proposed.[4,41,67] In vivo production of thrombopoietin by the liver (primary site) and the kidney (secondarily) is hypothesized to be constitutive. In this proposed model, changes in the endogenous level of circulating thrombopoietin reflect the degree of binding of thrombopoietin to platelets.[41] Megakaryocytes also bind thrombopoietin,[68] and therefore megakaryocyte mass also may be important in modulating the circulating thrombopoietin levels.[69] Elevation of either platelet or megakaryocyte mass would be expected to decrease plasma thrombopoietin below the level necessary to sustain basal megakaryocyte differentiation. Therefore, the decrease in polyploidization we observed after the increase in megakaryocyte and platelet mass was most likely the result of lowered endogenous thrombopoietin levels in these mice. Support for this interpretation is provided by studies in mice deficient in thrombopoietin ($TPO^{-/-}$), where the modal ploidy class is decreased to 8N and the megakaryocytes are smaller in size.[70]

In contrast to megakaryocyte maturation, no evidence for downregulation of megakaryocyte frequency is apparent after MGDF administration. This result is reminiscent of the response observed with 5-fluorouracil administration, where megakaryocyte ploidy and size were subject to different feedback regulatory mechanisms than proliferation.[35] These findings imply that megakaryocyte maturation may be more dependent than megakaryocyte precursor proliferation on thrombopoietin.

In our studies we found that a 25-μg/kg dose of MGDF was sufficient to maximally stimulate megakaryocyte ploidization and size. In contrast, stimulation of megakaryocyte proliferation was dependent on the dose we administered; the higher dose of MGDF gave a greater expansion of the 2N/4N megakaryocytic population. One interpretation is that less thrombopoietin is needed to induce ploidization and increase cell size than to stimulate proliferation by megakaryocyte precursors. However, we also observed that when less circulating thrombopoietin was presumably available (because of the increase in megakaryocyte and platelet mass), ploidization, but not frequency, was reduced. This paradox may be explained by location of megakaryocytes versus their precursors in the BM, and their accessibility to thrombopoietin. Mature megakaryocytes are known to lie adjacent to the BM sinus endothelium[71-73] and presumably have the most immediate access to circulating thrombopoietin. Thus, even at the lower dose of MGDF, megakaryocytes obviously had sufficient thrombopoietin binding to maximally stimulate ploidy and size. However, megakaryocyte precursors[73] are located distal to the sinus endothelium, and the effect of injected MGDF may be limited by its rate of diffusion from the vascular sinusoid to their distal location. Hence, a higher dose of MGDF was needed to penetrate the marrow and induce the greater degree of precursor proliferation. Therefore, the parasinusoidal location of mature megakaryocytes[72] makes them more susceptible to variations in circulating thrombopoietin levels than megakaryocyte precursors. Megakaryocyte number, unlike ploidy, did not decrease below control values on days 6 to 10 (Figs 2 and 3), when the thrombopoietin levels in the circulation were expected to be low. Because the megakaryocyte precursors are less reliant on circulating levels of thrombopoietin, they may be sustained by the low constitutive thrombopoietin production by marrow stromal cells.[19,74]

In summary, a single injection of PEG-rmMGDF significantly augments the proliferation of late megakaryocyte precursors and markedly stimulates megakaryocyte maturation. Although expansion of the proliferating 2N/4N cells appeared dependent on the dose of MGDF we administered, both 25-μg/kg and 250-μg/kg doses of MGDF were able to maximally enhance ploidization and cell size.

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RAPID COMMUNICATION

Oligodeoxynucleotides Antisense to the Proto-oncogene c-*mpl* Specifically Inhibit In Vitro Megakaryocytopoiesis

By Nassia Methia, Fawzia Louache, William Vainchenker, and Françoise Wendling

The proto-oncogene c-*mpl* encodes a protein whose sequence shares striking homologies with members of the highly conserved hematopoietin receptor superfamily. This gene had been transduced in a truncated form by the acute leukemogenic murine Myeloproliferative leukemia virus, which exhibits the unique property of inducing factor-independent proliferation and terminal differentiation of a broad spectrum of hematopoietic progenitors. Presently, the ligand and the role of c-*mpl* in the regulation of normal hematopoiesis are unknown. To show the function of c-*mpl*, its expression was first examined in human purified hematopoietic cell populations and, then, an antisense strategy was used. By RNA-based polymerase chain reaction, c-*mpl* transcripts were detected in purified CD34$^+$ cells, megakaryocytes, and platelets. Synthetic unmodified antisense oligodeoxynucleotides were derived from different regions of the c-*mpl* extracellular domain. On in vitro exposure of CD34$^+$ cells, two antisense oligomers led to a 50% to 70% inhibition of c-*mpl* mRNA synthesis, whereas their respective sense had no effect. Furthermore, the decrease in c-*mpl* mRNA correlated with a significant inhibition (range, 54% to 81%) of in vitro megakaryocytic colony formation (CFU-MK), whereas the growth of erythroid (BFU-E) or granulomacrophage (CFU-GM) colonies was unaffected. The data provide first evidences that c-*mpl* is involved in megakaryocytopoiesis. In addition, the results raise the possibility that this proto-oncogene encodes the receptor for a new cytokine specifically regulating thrombocytopoiesis.

RECEPTORS FOR interleukins (IL) and colony-stimulating factors (CSF) form a group of closely related glycoproteins (GPs) that define the hematopoietin receptor superfamily.[1-3] One oncogenic version of a member of this receptor superfamily had been naturally transduced in the genome of the highly leukemogenic murine Myeloproliferative Leukemia virus (MPLV).[4] We have previously shown that the MPLV envelope gene contains the oncogene v-*mpl* that displays unique biologic features. On in vivo or in vitro infection, murine multipotential as well as lineage-committed erythroblastic, megakaryocytic, myeloblastic, and mastocytic progenitors immediately acquire growth factor independence for both proliferation and terminal maturation.[5-7] In addition, evidence that the normal cellular proto-oncogene c-*mpl* plays a functional role restricted to hematopoiesis was suggested by the observation that its expression was found only in bone marrow (BM), spleen, or fetal liver from normal mice by Northern blot analysis, but not in other tissues.[7] Although these data clearly show that v-*mpl* was a potent disregulator of hematopoiesis, they did not provide any clue for the function of the normal proto-oncogene.

Using v-*mpl*–derived probes, a cDNA corresponding to the human c-*mpl* proto-oncogene was cloned. Sequence analysis predicts a protein that belongs to the highly conserved cytokine receptor superfamily.[8] However, neither its distribution in hematopoietic cells nor its ligand have been reported to date.

In an attempt to determine a role for c-*mpl* in regulating normal hematopoiesis, we have examined c-*mpl* expression by purified human hematopoietic cell populations and its function using an antisense strategy. We show here that c-*mpl* is expressed in CD34$^+$-purified cells, megakaryocytes, and platelets. Furthermore, we show that exposure of CD34$^+$ cells to c-*mpl* synthetic antisense oligodeoxynucleotides significantly inhibits the colony-forming ability of megakaryocytic progenitors (CFU-MK), but had no effect on erythroid (BFU-E) or granulomacrophage (CFU-GM) progenitors. Together, these data suggest that the c-*mpl*–encoded protein could be the receptor for a cytokine specifically regulating thrombocytopoiesis.

MATERIALS AND METHODS

Cell lines. Pluripotential (UT-7, TF-1, KU812, K153), erythro/megakaryocytic (Mo-7E, HEL, DAMI, Meg-01), myeloblastic (KG1a), promyelocytic (HL60), monocytic (U937), erythroleukemic (K562), and lymphoblastic B (Raji) and T (Mo or Jurkat) cell lines were maintained in α-Minimum Essential Medium (α-MEM) supplemented with L-glutamine (20 mmol/L), penicillin (100 U/mL), streptomycin (250 U/mL), and 10% heat-inactivated fetal calf serum (FCS).

Hematopoietic tissues and isolation of CD34$^+$ cells. Normal BM was obtained from hip surgery after informed consent for the procedure. Peripheral blood (PB) from healthy volunteers was collected on 100 U/mL preservative-free heparin. Experiments dealing with oligodeoxynucleotides were performed with CD34$^+$ cells isolated from frozen cytopheresis. They were obtained from patients

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with myeloma after hematologic recovery from high doses of chemotherapy for autografting.[9] Fetal liver (22 weeks of gestation) and thymus were obtained from therapeutic abortion after the guideline of the local Ethic Committee.

$CD34^+$ cells were isolated using immunomagnetic beads.[10] BM or blood mononuclear cells were separated over a Ficoll-metrizoate (Seromed, Biochrom KG, Berlin, Germany) gradient and depleted of monocytes by three cycles of plastic adherence at 37°C in Iscove's modified Dulbecco's medium (IMDM) containing 10% FCS. For isolation of $CD34^+$ cells, 50×10^6 adherence-depleted cells/mL were incubated for 10 minutes at 4°C in IMDM supplemented with 10% human antibody serum. Anti-CD34 monoclonal antibody (MoAb) (QBEND/10, obtained by courtesy of Dr Mel Greaves, Institute of Cancer Research, London, UK) was added at a final concentration of 1/2,500. After 1 hour exposure at 4°C with constant swirls, cells were washed and subsequently incubated for 1 hour at 4°C with magnetic beads coated with goat antimouse Ig (Dynabeads M-450, Dynal, Oslo, Norway) at a target cell to bead ratio of 1:5. Cells were detached free of beads by two exposures of 1.5 minutes each at 37°C in chymopapaine (Sigma, St Louis, MO, 150 U/mL in IMDM containing 0.01 mol/L EDTA). The percent yield of $CD34^+$ cells was in the range of 2.5% to 3% of the adherence-depleted population (0.5% to 1% of the initial cell count). This population was 95% to 98% pure as judged by labeling with a phycoerythrin-conjugated CD34 MoAb (HPCA-2, clone 8G2, Becton Dickinson, Mountain View, CA) after an overnight recovery in IMDM plus FCS.

Reverse transcription polymerase chain reaction (RT-PCR). Total cellular RNA (100 ng) or RNA[11] extracted from a pellet containing 1×10^5 $CD34^+$ cells were reverse-transcribed at 42°C for 40 minutes in a final volume of 50 μL with random hexanucleotides as primers: 28 μL RNA in H_2O, 5 μL Tris-HCL pH 8.3, 7 μL 1 mol/L KCl, 2 μL 0.25 mol/L $MgCl_2$, 2 μL 700 mmol/L 2β-mercaptoethanol, 2.5 μL 2 mmol/L deoxynucleoside triphosphate (dNTP) mix (deoxyadenosine [dATP], deoxycytidine [dCTP], deoxyguanosine [dGTP], and deoxythymidine [dTTP]), 1.5 μL hexanucleotides (100 ng/μL), 1 μL RNAsin (40 U/μL, Promega, Madison, WI), 1 μL avian myeloblastosis leukemia virus (AMV) reverse transcriptase (7.5 U/μL, Promega). To 10 μL of first-strand cDNA were added 90 μL of polymerase chain reaction (PCR) mix that contained: 10 μL 10× reaction buffer (100 mmol/L Tris-HCl pH 8.3, 15 mmol/L $MgCl_2$, 500 mmol/L KCl, and 0.1% gelatin), 10 μL of 2 mmol/L dNTP mix, 50 μL water, and 0.5 μL of Taq DNA polymerase (5 U/mL, Perkin Elmer Cetus, Norwalk, CT). Each 5' and 3' specific primers were added in a volume of 10 μL to give a primer concentration of 7 ng in the final 100 μL. The mixture was subjected to 35 amplification cycles using the Perkin-Elmer thermal cycler set as followed: denaturation at 94°C for 1 minute primer annealing at 55°C for 1 minute, extension at 72°C for 1.5 minutes.

Primers specific for the c-*mpl* message were: sense primer (nucleotide 843) 5'-TGGAGATGCAGTGGCACTTG-3' and antisense primer (nucleotide 1029) 5'-TGATGTCTGGGGTGTCAAGA-3'. Primers specific for the β2-microglobulin message[12] were: sense primer (nucleotide 1543) 5'-ACCCCCACTGAAAAAGATGA-3', antisense primer (nucleotide 3317) 5'-ATCTTCAAACCTCCATGATG-3'. PCR products (10 μL) were electrophoresed through a 2% agarose gel. Prehybridization was performed at 55°C for 4 hours and the amplification products detected by an overnight hybridization to synthetic ^{32}P-γ-ATP-labeled oligomer probes: c-*mpl* 5'-TTCTACCACAGCAGGGCACG-3', β2-microglobulin 5'-GCCCAAGATAGTTAAGTGGG-3'. Primers specific for the c-*kit* message were those published by Ratajczak et al.[13] Filters were washed twice for 2 minutes at room temperature and then for 15 minutes at 60°C in 2× standard saline citrate (SSC)/0.1% sodium dodecyl sulfate (SDS). Autoradiographs were exposed for 6 hours at −80°C with intensifying screens.

Antisense oligodeoxynucleotide synthesis and cell treatment. Unmodified 18-mer oligodeoxynucleotides were synthesized on a DNA synthesizer (model 380 B; Applied Biosystems, Foster City, CA), precipitated and resuspended in IMDM as previously described.[14] c-*mpl* antisense AS3 (5'-GGCCCAGGAGGGCATCTT-3') and AS6 (5'-TGCTGTCAGAGCTGAAGC-3') correspond to nucleotides −3 to +15 or +690 to +708, respectively. Antisense AS1, AS2, and AS4 correspond to nucleotides +1 to +18, +22 to +39 and +13 to +30, respectively. AS7 was a scrambled antisense to AS6.

$CD34^+$ cells were incubated at a concentration of 1×10^6 cells/mL in serum-deprived medium as described.[14] Medium (IMDM) contained iron-saturated human transferrin (300 μg/mL), insulin (100 ng/mL), calcium chloride (28 μg/mL), deionized bovine serum albumin (BSA) (2%), 20 μL/mL of a sonicated lipid mixture (7.8 mg cholesterol, 6.14 mg oleic acid, and 7.4 mg dipalmitoyl lecithin in 10 mL IMDM). Incubation medium was supplemented with recombinant human (r-hu)IL-3 (100 U/mL), r-hu granulocyte-macrophage CSF (GM-CSF) (2.5 ng/mL), r-hu G-CSF (100 ng/mL) and r-hu erythropoietin (EPO) (2 U/mL). Oligonucleotides were used at a concentration of 10 mmol/L (70 μg/mL). After 16 hours incubation at 37°C, 5 mmol/L oligonucleotides were added. Cells were further incubated for additional 6 hours and then washed in IMDM before plating or RNA extraction.

Colony assays. Cells were plated in the fibrin clot culture system as described.[15,16] For BFU-E– or CFU-GM–derived colonies, IMDM medium contained 20% preselected FCS (Boehringer, Mannheim, Germany), 1% deionized BSA (Cohn fraction V, Sigma), L-asparagine (20 μg/mL), $CaCl_2$ (28 μg/mL), 10% bovine citrated plasma (BCP; GIBCO, Paisley, Scotland), r-hu IL-3 (100 U/mL), r-hu GM-CSF (2.5 ng/mL), r-hu G-CSF (100 ng/mL), and r-hu Epo (2 U/mL). Cultures (four plates per point) were performed at a concentration of 200 cells in a volume of 0.5 mL (Linbro plates CV-TC 96, Flow Laboratories, Irvine, CA). Cultures were harvested onto slides after 12 days incubation. Clots were fixed, stained with benzidine, counterstained with hematoxylin, and the number of colonies (more than 50 cells) counted.

For CFU-MK–derived colonies, cells were seeded at a concentration of 500 cells/0.5 mL in culture containing either 10% human serum derived from platelet poor plasma (PPP) + r-hu IL-3 (100 U/mL) or 10% serum from aplastic patients (SA) + r-hu IL-3 (100 U/mL). Cultures were incubated for 12 days. To ensure an accurate scoring of this colony type, fibrin clots were harvested onto slides, fixed for 5 minutes with 10% neutral formalin and reacted with Y2-51 anti GpIIIa (CD61) MoAb for 30 minutes. Fixation of MoAb was shown using mouse 1/100 diluted IgG + IgM (Fab')2 coupled to alkaline phosphatase (Caltag laboratories, San Francisco, CA) as described.[17]

RESULTS

c-mpl expression by human leukemic cell lines. We first used various human leukemic cell lines to investigate c-*mpl* expression by the RNA-based PCR technique. Reverse transcription was performed on 100 ng total RNA with random hexanucleotides as primers. The resulting cDNAs were amplified using primers specific for a 220-base sequence of the c-*mpl* message and, in parallel, with primers specific for a 116-base sequence of the β2 microglobulin message to act as an internal control for each sample. c-*mpl* transcripts were detected in UT-7, Mo-7E, TF-1, HEL, DAMI,

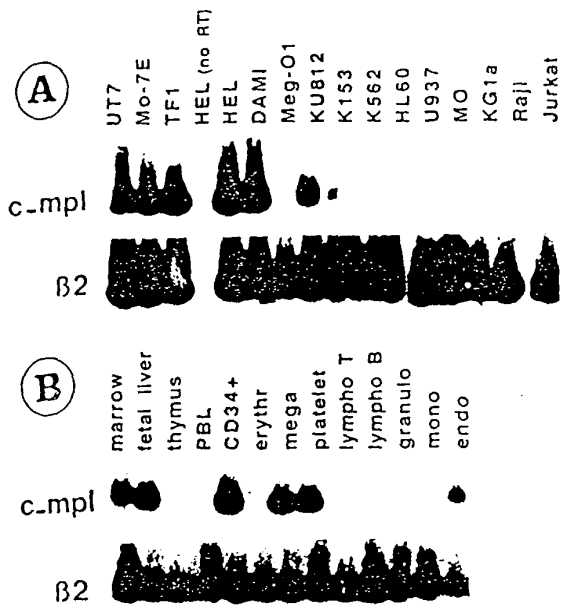

Fig 1. Expression of c-*mpl* and β-2 microglobulin (β2) by human leukemic cell lines (A) and normal purified hematopoietic cell populations (B). RT-PCR was performed on 100 ng total RNA prepared from pluripotential (UT-7, TF-1, KU812, Mo-7E), erythro/megakaryocytic (HEL, DAMI, Meg-01, K153), myeloblastic (KG1a), promyelocytic (HL60), monocytic (U937), erythroleukemic (K562), lymphoblastic B (Raji) and T (Mo or Jurkat) cell lines, hematopoietic tissues, or purified subpopulations. For the $CD34^+$ population, RNA was extracted from a pellet containing 1×10^5 cells. Reverse-transcription and amplification were performed as described in Materials and Methods section. β2 microglobulin transcripts served as internal controls for each sample. Autoradiographs were exposed for 6 hours at −80°C with intensifying screens.

KU812, and faintly in K153 cells, but not in Meg-01, K562, HL60, U937, MO, KG1 a, Raji, or Jurkat cells (Fig 1A). Most PCR-positive cell lines expressed spontaneously and unambiguously, in addition to myeloid and erythroid specific antigens, the platelet GPs GpIIb and GpIIIa indicating that they represent cells in which commitment to the megakaryocytic lineage of differentiation had occurred.[18] Negative cell lines, with the exception of Meg-01, presented phenotypes of cells committed either to the erythroblastic, myeloblastic, monocytic, or lymphoblastic lineages. Northern blot analysis of total RNA showed the major 3.7-kb c-*mpl* mRNA in HEL[8] and DAMI cells, but not in the other PCR-positive cell lines (data not shown). This was in accordance with semiquantitative PCR analysis showing that c-*mpl* transcripts were detectable on RNA prepared from only 10 HEL or DAMI cells, whereas a minimum of 500 UT-7 or TF-1 cells were necessary to show a signal.

c-mpl expression by purified human hematopoietic cell populations. Expression of c-*mpl* was then investigated in normal human hematopoietic cells by RT-PCR, because mRNAs of most hematopoietic cytokine receptors are usually found in low abundance. Signals consistent with c-*mpl* amplified transcripts were detected in unseparated BM, fetal liver, and faintly in PB leukocytes (PBL), but not in thymus (Fig 1B). To further document which subpopulations of hematopoietic cells expressed c-*mpl*, we purified progenitor and precursor cells from BM of normal adult donors and we separated the mature nucleated cells from PBL. $CD34^+$ cells were isolated using magnetic beads.[10] This population, which represents approximately 1% of total BM cells, is enriched in primitive and committed progenitors from all lineages.[19] Erythroblasts (2×10^6 cells) were obtained from pure BFU-E-derived colonies plucked from methylcellulose after 10 days in culture. Megakaryocytes (6×10^6 cells) were isolated from day-12 liquid cultures[20] by use of the Y2-51 anti GpIIIa (CD61) MoAb and magnetic beads. This population comprised more than 85% megakaryocytes, the majority of contaminating cells being macrophages. Platelets were obtained from plasma of fresh blood cleared of leukocytes by filtration (1×10^{11} platelets that were contaminated by 3×10^5 leukocytes). PBL were separated into T or B lymphocytes, granulocytes, and monocytes by panning with MoAbs or adherence, respectively. Endothelial cells were cultured from umbilical cord. Strong signals corresponding to the c-*mpl* message were only detected in purified $CD34^+$ cells, highly enriched megakaryocytes, and platelets (Fig 1B). A mild signal was also seen on endothelial cells, whereas the other cell populations were negative. PCR was next performed on RNA from $CD34^+$ cells further purified, by two-color flow cytometry, into a $CD38^{null}$ population depleted in most committed clonogenic progenitors but enriched in primitive stem cells and a $CD38^+$ population that contained clonogenic progenitors.[21] Expression of c-*mpl* was detected in both populations; however, the signal obtained with $CD34^+/CD38^{null}$ population was 10 to 50 times more intense than in the $CD34^+/CD38^+$ population (data not shown). The high level of transcripts found by PCR in the highly enriched populations of megakaryocytes and platelets was confirmed by Northern analysis where the 3.7-kb c-*mpl* mRNA could be shown (data not shown). These results indicate that c-*mpl* is expressed in hematopoietic progenitors during the very primitive stages and in cells from the megakaryocytic lineage where transcripts remain detectable until the latest stage of maturation.

Degradation of c-mpl *mRNA by antisense oligodeoxynucleotides.* To directly determine the role of c-mpl in regulating normal hematopoiesis, we exposed $CD34^+$ cells purified from cytopheresis to antisense oligodeoxynucleotides. This strategy has been successfully used to address the function of proto-oncogenes or growth factor receptors in hematopoiesis.[22-24] At least two different mechanisms are involved in the effects of antisense oligomers in cells: formation of oligonucleotide/RNA duplex results in a degradation of mRNA through RNAse H or in an inhibition of translation without altering RNA stability.[25,26] To determine the stability of c-*mpl* transcripts and the half-life of the protein, we exposed DAMI cells to actinomycin D (5 μg/mL) for varying intervals. C-*mpl* mRNA was stable for about 30 minutes after actinomycin D addition and then destabilized. This treatment resulted in a total disappearance of the c-*mpl* protein after 8 hours as determined by labeling these cells with a MoAb directed to c-*mpl* extracel-

lular domain (MoAb M1, obtained from Immunex Corp, Seattle, WA) (data not shown).

$CD34^+$ cells were incubated in serum-free medium containing growth factors and synthetic unmodified 18-mer sense, antisense, or scrambled oligodeoxynucleotides. Equal numbers of cells (1×10^5 cells) were used for total RNA extraction and subsequent PCR analysis; the remaining cells were seeded in cultures to assess the biologic effect of oligodeoxynucleotides treatment. Six oligodeoxynucleotides complementary to different regions of the first c-*mpl* extracellular domain were derived. To determine whether any of them would result in c-*mpl* mRNA degradation, samples of the PCR reaction were removed after three different numbers of cycles to monitor that amplification remained within the exponential phase. Two oligodeoxynucleotides, antisense 3 (AS3) encompassing the first methionine codon and antisense 6 (AS6) complementary to a sequence present in the peculiar extraglobular domain,[8] produced a significant decrease in c-*mpl* mRNA levels. In contrast, their respective sense (S3 and S6), a scrambled antisense to AS6 (AS7) or the other antisense oligomers had no effect (Fig 2). Controls for RNA integrity and efficient reverse-transcription were given by amplification of $\beta 2$ microglobulin transcripts. In addition, we also amplified the c-*kit* message expressed by primitive and committed hematopoietic progenitors[13] to test for the specific action of the oligonucleotides used. No decrease in $\beta 2$ microglobulin nor in c-*kit* messages was found. These data suggested that antisense 3 and 6 bind specifically to c-*mpl* mRNA resulting in its degradation.

Suppression of CFU-MK colony formation by exposure of $CD34^+$ cells to c-mpl *antisense oligodeoxynucleotides.* The biologic significance of a decrease in c-*mpl* mRNA was examined by plating oligomers-treated $CD34^+$ cells in fibrin clot cultures under optimal conditions.[15,16] As shown in Fig 3 (four separated experiments performed with progenitors isolated from three different donors), exposure of $CD34^+$ cells to sense, antisense, or scrambled oligomers did not affect the development of BFU-E– or CFU-GM–derived colonies (Student's *t*-test: $.400 > P > .050$), nor colony size or maturation compared with untreated controls. To determine the influence of oligomers treatment on megakaryocyte colony formation (CFU-MK), oligomers-treated cells were plated in medium containing either human serum derived from PPP + r-hu IL-3 to ensure optimal culture conditions or serum from patients with aplastic marrow (SA) as a rich source of MK colony-stimulating activity (Meg-CSA)[28] + r-hu IL-3 to stimulate both CFU-MK proliferation and maturation. Because CFU-MK produce colonies containing highly variable numbers of mature MK (from 5 to >100 cells), cultures were incubated with the Y2-51 MoAb specific for GpIIIa and MoAb fixation was shown by the immunoenzymatic alkaline phosphatase antialkaline phosphatase labeling to unambiguously visualize this colony type.[17] Combined results from four experiments are shown in Fig 4. Whatever the culture conditions (PPP + IL-3 or SA + IL-3), antisense AS3 and AS6, which decreased c-*mpl* mRNA, resulted in a statistical and significant reduction of MK colony numbers whereas their respective sense (S3 and S6) or the scrambled antisense to AS6 (AS7) had no detectable effect. In experiments where cells were grown with SA + IL-3, the decrease in CFU-MK–derived colonies was 54%, 58%, 62%, and 74% for AS3 and 54%, 70%, 76%, and 81% for AS6 (Student's *t*-test: $.005 > P > .001$), in comparison with cells exposed to the respective sense oligonucleotides. The percent inhibition in MK colony formation in cultures containing PPP + IL-3 was in the same order of magnitude. In contrast, the antisense AS1 and AS2, their respective sense S1 and S2 or the scrambled AS7 oligomers did not significantly affect CFU-MK development nor mean numbers of MK per colony when compared with untreated controls. At the cell concentration used (500 cells/plate), only rare MK colony developed in the absence of r-hu IL-3. Together the data show that the decrease in c-*mpl* mRNA on exposure of $CD34^+$ cells to antisense AS3 and AS6 correlates with a profound inhibition of CFU-MK development, whereas BFU-E or CFU-GM colony formation was not affected.

DISCUSSION

These studies provide the first evidences for a function of the cytokine receptor encoded by the c-*mpl* gene. Because a ligand for this receptor is still unknown, we examined expression and distribution of this receptor by RT-PCR. Using various human leukemic cell lines representing cells arrested at different stages of lineage development, we ob-

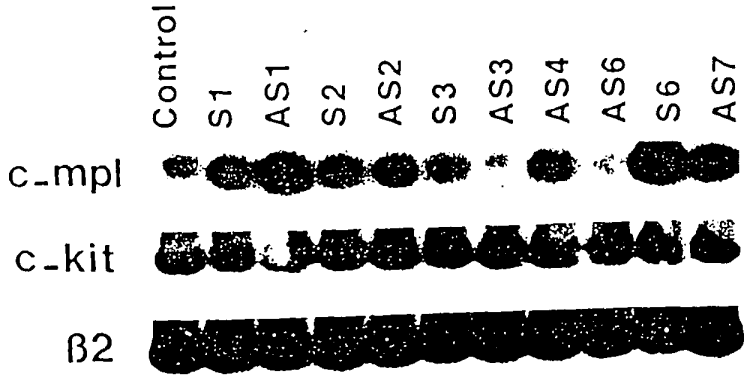

Fig 2. Level of c-*mpl* mRNA after exposure of blood-derived $CD34^+$ cells to sense, antisense, or scrambled antisense oligodeoxynucleotides. $CD34^+$ cells from cytopheresis were incubated for 22 hours in serum-free medium with r-hu growth factors and unmodified 18-mer oligonucleotides at a final concentration of 10 mmol/L (70 μg/mL). RNA was extracted from 1×10^5 cells, reverse-transcribed, and amplification was performed for 32 cycles. $\beta 2$ microglobulin and c-*kit* transcripts indicate the specificity of c-*mpl* antisense oligomers used. Autoradiographs were exposed for 6 hours at −80°C with intensifying screens.

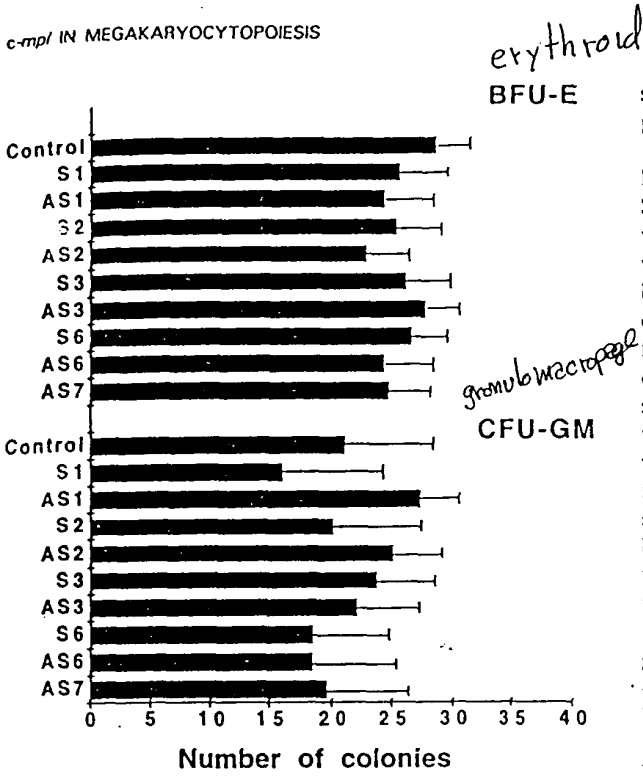

Fig 3. Effect of oligodeoxynucleotides treatment of blood-derived CD34$^+$ cells on in vitro BFU-E and CFU-GM colony formation. Cells were plated in the fibrin clot culture system at a concentration of 200 cells in a volume of 0.5 mL in medium containing 20% preselected FCS and r-hu IL-3 (100 U/mL), r-hu GM-CSF (2.5 ng/mL), r-hu G-CSF (100 ng/mL), and r-hu Epo (2 U/mL). Cultures were harvested after 12 days incubation, stained with benzidine, counterstained with hematoxylin, and the number of colonies (more than 50 cells) counted. Data are mean colony numbers ± SD of four different combined experiments (four plates/point/experiments).

served that c-*mpl* transcripts were detectable in pluripotent factor-dependent cell lines (UT-7, TF1, Mo-7E) and in several cell lines (HEL, DAMI, KU812, K153) that, among other lineage-specific antigens, spontaneously expressed both the GPIIb and GPIIIa platelet GPs.[18] Because of the low abundance of cytokine receptor mRNA in normal hematopoietic cells, we applied the RT-PCR technique to determine which subpopulations within the hematopoietic system expressed the c-*mpl*–encoded receptor. Our data indicated that transcription of c-*mpl* occurred in a cell fraction enriched in very primitive hematopoietic progenitor cells, the CD34$^+$/CD38^{null} population.[21] C-*mpl* transcripts were also detected in lineage-committed CD34$^+$/CD38$^+$ progenitors. However, when the presence of the c-*mpl* message was studied in purified erythroblastic precursor cells or in purified mature cells from various lineages, we found that expression was restricted to cell populations highly enriched in MK or platelets. These data indicate that the c-*mpl*–encoded receptor is expressed in early hematopoietic cells, but its expression appears to be downregulated during the various pathways of differentiation except for the megakaryocytic lineage. They also suggested that c-*mpl* could serve in the processes of MK proliferation, maturation, and platelets production.

To further determine whether c-*mpl* played a role in regulating megakaryocytopoiesis, we used an antisense strategy. Six unmodified 18-mer antisense oligodeoxynucleotides were derived that corresponded to sequences contained within the first c-*mpl* extracellular domain.[8] Two of them, but not their respective sense or a scrambled antisense, decreased c-*mpl* mRNA level in treated CD34$^+$ cells isolated from PB (Fig 2). The specificity of these antisense oligodeoxynucleotides was given by the following criteria: (1) No significant decrease in β2 microglobulin or c-*kit* messages was observed in oligomer-treated cells, and (2) no homology with other members of the cytokine superfamily could be found when the oligomer sequences were compared with sequences present in the European Molecular Biology Library data bank. Furthermore, exposure of CD34$^+$ cells to the antisense oligodeoxynucleotides that degradated c-*mpl* mRNA resulted in a profound inhibition of in vitro CFU-MK colony formation (range = 54% to 81%) whereas the growth of BFU-E or CFU-GM was unaffected (Figs 3 and 4). These results indicate that the c-*mpl*–encoded protein likely transduces signal(s) for survival, proliferation, and/or maturation in MK progenitors. Furthermore, because inhibition of CFU-MK development was observed in cultures

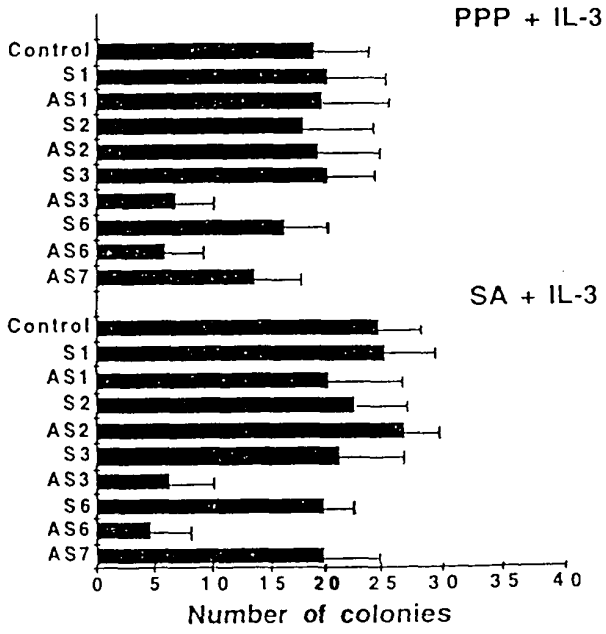

Fig 4. Effect of oligodeoxynucleotides treatment of blood-derived CD34$^+$ cells on in vitro CFU-MK colony formation. Cells were seeded at a concentration of 500 cells/0.5 mL in fibrin culture containing either 10% human serum derived from PPP + r-hu IL-3 (100 U/mL) or 10% serum from aplastic patients (SA) + r-hu IL-3. Clots were harvested onto slides after 12 days of growth, fixed with neutral formalin and reacted with the mouse Y2-51 anti GpIIIa MoAb. Fixation of MoAb was shown using mouse IgG + IgM (Fab')2 coupled to alkaline phosphatase. Data represent mean colony numbers ± SD of four different combined experiments (four plates/point/experiments).

containing normal human serum or serum from aplastic patients and r-hu IL-3, it is tempting to hypothesize that promotion of MK colony formation occurred through a synergistic activation of c-*mpl* and IL-3 receptors. As a consequence, this would imply that the c-*mpl* ligand must be present in serum or synthesized by $CD34^+$ cells.

Platelet production is regulated at multiple cellular levels by a large number of cytokines such as IL-3, GM-CSF, IL-6, leukemia inhibiting factor (LIF), Steel factor (SCF), Epo, or IL-11.[27-33] At least in vitro, none of these cytokines is lineage specific. Nevertheless, it is known that each one of them stimulated MK colony formation and/or MK maturation in synergy with either IL-3 or GM-CSF. The α and β chains cDNAs making up high-affinity receptors for these different cytokines have been cloned, with the exception of the receptor for IL-11. Thus, the possibility that c-*mpl* may encode a component of the IL-11 receptor complex cannot be totally eliminated. However, such an hypothesis seems very unlikely for the following reasons: (1) it was reported that IL-11 is a potent stimulator of erythropoiesis, capable of stimulating BFU-E and supporting the maturation of erythroblasts.[34] If these observations are indeed caused by a direct effect of IL-11 on these populations, then our research of c-*mpl* transcripts in purified erythroblasts by the highly sensitive PCR technique should have shown signals, (2) in addition, the experiments dealing with antisense oligodeoxynucleotides would have shown an inhibition of the growth of BFU-E–derived colonies, and (3) recent data indicate that the signal transducer of the IL-11 receptor is GP 130.[35]

It has been suggested for many years that serum (normal or from thrombocytopenic animals and human) contained a unique growth factor that synergized with various cytokines to promote the growth and the maturation of megakaryocytes.[36-39] It is also now known that this synergistic factor in serum is distinct from IL-1α, IL-3, IL-4, IL-6, IL-11, SCF, Epo, G-CSF, and GM-CSF.[40] However, although numerous investigators have attempted to isolate this activity, no recombinant molecule is yet available to perform experiments aiming to determine whether or not it would bind to c-*mpl*.[41] At present, it is not known either whether c-*mpl* represents a receptor chain capable of both binding a ligand and transducing a signal or the β component of a cytokine receptor complex. Biochemical characterization of the c-*mpl* receptor, isolation, and molecular cloning of its ligand are now necessary to understand which of these possibilities is correct and to allow further investigations.

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Murine c-*mpl*: a member f th hemat poietic growth factor r c ptor sup rfamily that transduc s a proliferative signal

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The murine myeloproliferative leukemia virus has previously been shown to contain a fragment of the coding region of the c-*mpl* gene, a member of the cytokine receptor superfamily. We have isolated cDNA and genomic clones encoding murine c-*mpl* and localized the c-*mpl* gene to mouse chromosome 4. Since some members of this superfamily function by transducing a proliferative signal and since the putative ligand of mpl is unknown, we have generated a chimeric receptor to test the functional potential of mpl. The chimera consists of the extracellular domain of the human interleukin-4 receptor and the cytoplasmic domain of mpl. A mouse hematopoietic cell line transfected with this construct proliferates in response to human interleukin-4, thereby demonstrating that the cytoplasmic domain of mpl contains all elements necessary to transmit a growth stimulatory signal. In addition, we show that 25–40% of *mpl* mRNA found in the spleen corresponds to a novel truncated and potentially soluble isoform of mpl and that both full-length and truncated forms of mpl protein can be immunoprecipitated from lysates of transfected COS cells. Interestingly, however, although the truncated form of the receptor possesses a functional signal sequence and lacks a transmembrane domain, it is not detected in the culture media of transfected cells.

Key words: alternative splicing/chimeric receptor/chromosomal mapping/c-*mpl*/cytokine receptor

Introduction

Mpl is a member of the cytokine receptor superfamily for which no ligand has as yet been identified. A portion of the c-*mpl* gene was originally discovered fused to viral sequences encoding the envelope protein of a mutant Friend murine leukemia virus (Souyri *et al.*, 1990). This mutant strain was called myeloproliferative leukemia virus (MPLV) because it caused a broad spectrum of myeloid leukemias in mice including erythroid, granulocytic, monocytic, megakaryocytic and mast cell leukemias (Wendling *et al.*, 1986, 1989).

The human homolog of v-*mpl* has been shown to have sequence homology to members of the cytokine receptor superfamily (Vigon *et al.*, 1992), which is characterized by a common structural design f the extracellular domain, with four conserved cysteine residues in the N-terminal portion, and a short motif, tryptophan-serine-x-tryptophan-serine (WSXWS), located proximal to the transmembrane domain (Bazan, 1990). All but 40 amino acids of the extracellular domain of mpl, including the conserved cysteines, were deleted in the fusion protein encoded by v-*mpl* and replaced by viral envelope sequences. The WSXWS motif, the transmembrane domain and the entire cytoplasmic domain of mpl were preserved (Souyri *et al.*, 1990). By Northern analysis, mpl was found to be expressed only in hematopoietic tissue, spleen, bone marrow and fetal liver. No expression was detected in thymus.

Members of the cytokine receptor superfamily may be grouped into three functional categories (for review see Nicola and Metcalf, 1991): single chain receptors, such as erythropoietin receptor (EPO-R), granulocyte colony stimulating factor receptor (G-CSF-R) or interleukin-4 receptor (IL4-R), bind ligand with high affinity via the extracellular domain and also generate an intracellular signal. A second class of receptors, so-called α-subunits, includes interleukin-6 receptor (IL6-R), granulocyte-macrophage colony stimulating factor receptor (GM-CSF-R), interleukin-3 receptor (IL3-Rα) and other members of the cytokine receptor superfamily. These α-subunits bind ligand with low affinity but cannot transduce an intracellular signal. A high affinity receptor capable of signalling is generated by a heterodimer between an α-subunit and a member of a third group of cytokine receptors, termed β-subunits, e.g. β_c, the common β-subunit for the three α-subunits IL3-Rα, IL5-Rα and GM-CSF-R. It is not known whether mpl is capable of transducing a signal, or whether mpl can bind a ligand. The hematopoietic lineages in which mpl is physiologically active have not been determined, but the wide range of leukemias caused by v-mpl suggests that this receptor may affect multiple myeloid lineages and/or act at the level of a myeloid progenitor.

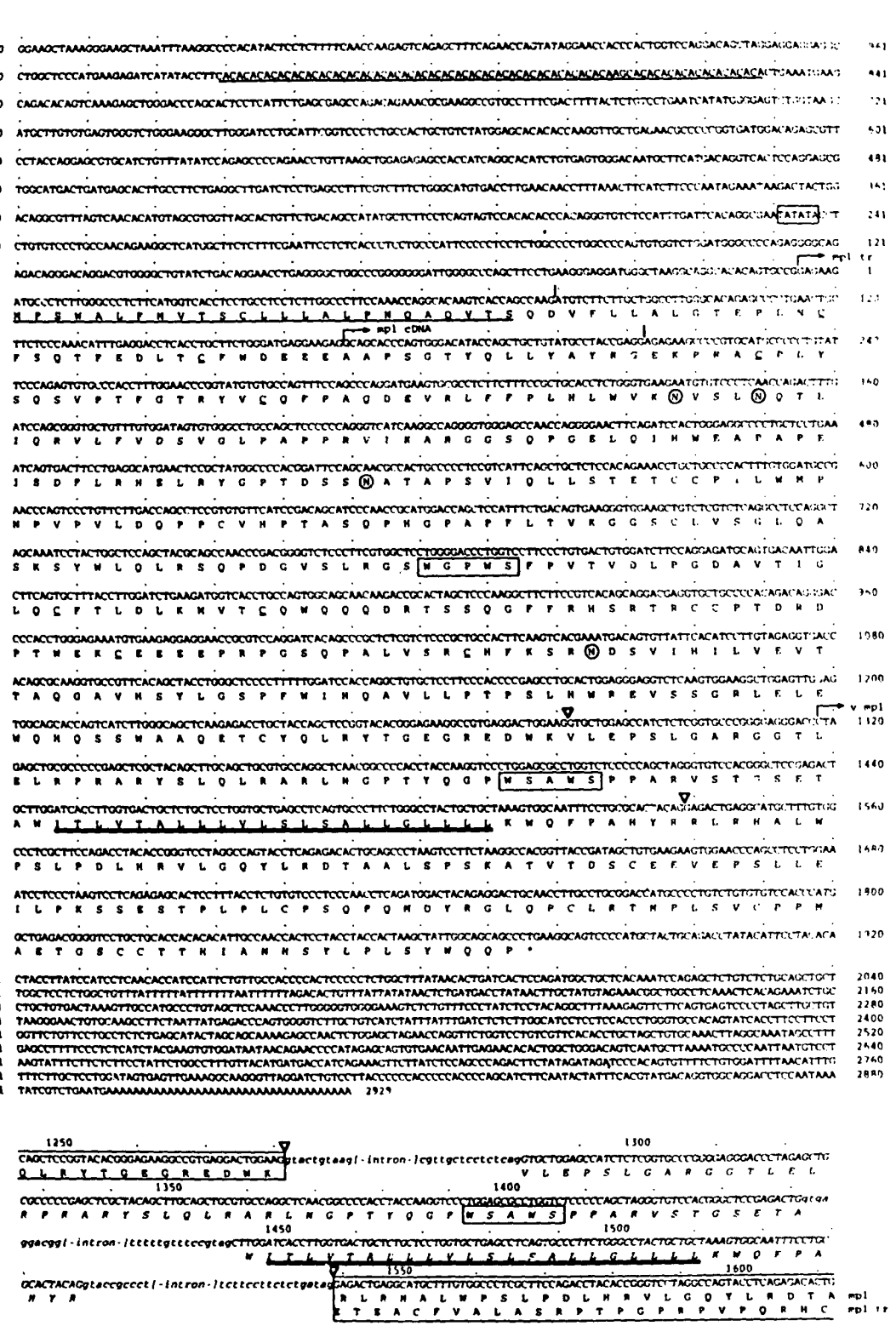
Here we present the full structure of the putative murine mpl transmembrane receptor and a potentially soluble isoform. Further, using a chimeric receptor construct we show that the cytoplasmic portion of mpl contains the elements necessary to transduce a proliferative signal in murine hematopoietic cells. Our results indicate that mpl is either a single chain receptor or a signal transducing β-subunit and suggest that v-mpl may function as a constitutively activated receptor.

Results

Molecular cloning of mouse mpl

We isolated 14 independent clones encoding mpl from an unamplified mouse spleen cDNA library using a 590 bp probe representing the entire v-*mpl* sequence. The clones can be grouped into three classes. The first represents clones coding for mpl, a transmembrane protein. The longest cDNA clone of this class starts 165 bp 3' of the putative initiator codon ATG (Figure 1A), as defined by the genomic c-*mpl*

clone (see below). The size of the full length composite *mpl* cDNA (2930 bp) is consistent with the reported size of 3 kb for mouse *mpl* mRNA on Northern blots (Souyri *et al.*, 1990). The open reading frame encodes a protein of 625 amino acids with a calculated mol. wt of 69 817, including a putative signal peptide of 25 amino acids. The extracellular



portion of the encoded protein is composed of two subdomains, each with four conserved cysteines and a WGXWS or a WSXWS motif. A hydrophobic transmembrane segment of 22 amino acids is f llowed by a 120 amino acid cytoplasmic domain. In addition to this clone, five shorter clones with identical sequence were isolated.

Clones of the second class encode a truncated form of mpl, designated mpl-tr. The longest of these cDNA clones contains the putative initiator codon ATG (Figure 1A). *Mpl-tr* is identical in sequence to *mpl* except for a deletion of 257 bp of the cDNA coding for the 54 C-terminal amino acids of the extracellular domain including the conserved WSXWS motif as well as the transmembrane domain and the first eight amino acids of the cytoplasmic domain (Figure 1A). This deletion generates a frame shift, and the reading frame terminates after 30 amino acids (Figure 1B). Mpl-tr cDNA encodes a protein of 457 amino acids including the signal peptide, with a calculated mol. wt of 51 162. A total of four clones were isolated that possess the identical deletion. On Northern blots only a single band was detected. However, this method does not resolve the difference of 257 nucleotides between *mpl* and *mpl-tr* transcripts (see below).

The third class of clones represents unspliced pre-mRNA. Three overlapping clones were sequenced across the 3' portion of the region deleted in *mpl-tr* (Figure 1B). The sequence at this 3' boundary agrees with intron/exon consensus sequences (Shapiro and Senapathy, 1987). Thus, the region of 257 bp deleted in *mpl-tr* represents two exons of 161 bp and 96 bp encoding the WSXWS motif and the transmembrane domain, respectively, interrupted by an intron of 160 bp.

The 5' boundary of the excluded sequence was defined by sequencing a mouse c-*mpl* genomic clone. Using an *Eco*RI–*Stu*I fragment representing the 5' end of the *mpl* cDNA to screen a mouse genomic DNA library, we isolated a 16 kb genomic fragment that encompasses the excluded region. This genomic sequence is identical to the cDNA sequence up to the 5' boundary of the deleted region, where it diverges from the cDNA sequence and displays a splice donor consensus (Figure 1B). Thus, the truncated form is generated by excluding two exons.

In order to confirm that the first ATG in *mpl-tr* cDNA is the initiation codon we sequenced the corresponding region of the genomic clone (Figure 1A). Upstream of the putative initiation codon we found stop codons in all three reading frames. The sequence surrounding this ATG agrees with the Kozak consensus (Kozak, 1989).

Chromosomal localization of c-mpl

The presence of a polymorphic CA-dinucleotide repeat just 5' of the c-*mpl* gene readily permitted us to locate this gene on the mouse genetic map. This was done using two approaches: typing the strain distribution patterns (SDP) of c-*mpl* in recombinant inbred (RI) strains (Taylor, 1989) (Table I) and using an interspecific backcross of (C57BL/6 × *Mus spretus*) × *Mus spretus* (Table II). The CA-dinucleotide repeat is located in the genomic c-*mpl* sequence 931 bp upstream of the putative initiator ATG (Figure 1A). Oligonucleotides flanking this repeat amplify a 205 bp product from C57BL/6J DNA, a 185 bp product from DBA/2J DNA and a 195 bp product from *M.spretus* DNA that can be separated by electrophoresis on 1.4% agarose gels or 8% polyacrylamide gels (not shown). This polymorphism was used to type the strain distribution pattern of c-*mpl* in the C57BL/6J × DBA/2J (BXD) RI strains (Table I). By this approach, c-*mpl* maps to mouse chromosome 4 between the previously mapped markers *Pmv-19* and *D4Mit12*. The CA repeat polymorphism was also used to type the strain distribution pattern of c-*mpl* in an interspecific backcross of (C57BL/6 × *M.spretus*) × *M.spretus* (Table II) and confirmed the location on chromosome 4, between *Pmv-19* and *D4Mit11*. The

Table I. Strain distribution pattern of c-*mpl* polymorphisms among BXD recombinant inbred mice

	BXD																							
	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
Pmv-19	D	B	D	B	B	D	B	D	B	D	D	B	D	D	B	D	D	D	B	D	D	D	D	D	B	D
Mnr-13	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	D	B	D	D	D	D	D	B	D
Cyp-4a	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	D	B	D	D	D	D	D	B	D
Glut-1	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	D	B	D	D	D	D	D	B	D
Ms15-1	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	D	B	D	D	D	D	D	B	D
c-*mpl*	D	B	D	B	B	D	B	D	B	D	D	D	D	D	B	D	D	D	B	D	D	D	D	D	B	D
D4Mit12	D	B	D	B	D	D	B	D	D	D	D	D	D	D	B	D	B	D	B	D	D	D	D	D	B	D

The SDPs of c-*mpl* and flanking markers on chromosome 4 are shown. Strains carrying the C57BL/6J allele are denoted by a B, and those carrying the DBA/2J allele are denoted by a D. Recombination events are indicated by a line. There are no recombinants between *Mnr-13*, *Cyp-4a*, *Glut-1*, *Ms15-1* and c-*mpl*.

Fig. 1. (A) Composite genomic and cDNA sequence for c-*mpl*. The sequence shown is composed of genomic sequence (position −1080 to +216) and of cDNA sequence (position −6 to +2929) for c-*mpl*. The sequence of the overlapping region from −6 to +216 was identical. The position of two introns is indicated by vertical lines. The 5' end of the longest cDNA clone for mpl-tr and mpl and the beginning of the sequence captured in v-*mpl* are indicated by arrows. The cDNA for mpl-tr contains the putative initiator ATG. The cDNA for mpl is missing 165 bp at the 5' end. The boundaries of the deletion in the mpl-tr cDNA are marked by triangles. Thin lines demarcate the CA-dinucleotide repeat and the putative signal peptide; the thick line indicates the transmembrane domain. The putative TATA-box and the conserved WSXWS and WGXWS motifs are boxed, and the conserved cysteines are underlined. The four potential N-glycosylation sites are circled. (B) Intron/exon structure of the region deleted in *mpl-tr*. The numbers refer to the nucleotide position as in (A). The 5' and 3' boundaries of the deleted region are boxed. The region deleted in mpl-tr is italicized. The exon/intron junction at the 5' boundary was determined by sequencing a genomic clone for c-*mpl*. The 3' boundary and the two exons coding for the region deleted in mpl-tr are derived from sequence of unspliced cDNA clones. The two reading frames utilized in mpl and *mpl-tr* are shown for the 3' boundary.

Table II. Recombination frequencies of c-*mpl* with flanking markers in an interspecific backcross

Interval	R	N	Recombination % ± SE	Confidence 95% limits
D4Mit9–c-*mpl*	12	91	13.2 ± 3.55	7.0–21.9
c-*mpl*–*D4Mit11*	3	92	3.26 ± 1.85	0.7–9.2

Percentage recombination between markers, standard error and confidence limits were calculated on RI manager version 2.3 (Manly *et al.*, 1991) from the number of recombinants (*R*) in a sample size (*N*).

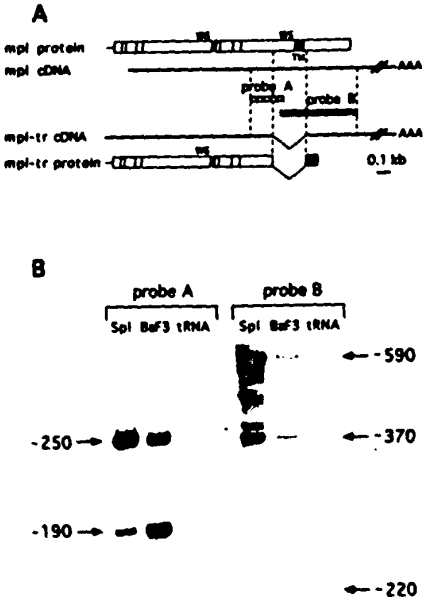


Fig. 2. (A) The position and length of the two RNase protection probes A and B, as used in (B), are shown. The two longest cDNA clones for mpl and mpl-tr are drawn to scale and aligned with their deduced protein products. Boxes represent the domains of the mpl protein. The position of each of the four conserved cysteines is indicated by a vertical line, 'WG' and 'WS' show the position of the conserved WGXWS and WSXWS motifs respectively. The deletion in the central portion of the *mpl-tr* cDNA and in the corresponding mpl-tr protein region is indicated by the thin line. This causes a frame shift and the mpl-tr protein terminates prematurely after 30 amino acids as indicated by the stippled box (see also Figure 1B). (B) Ribonuclease protection assay with *mpl* antisense RNA probes. Numbers indicate the approximate length of protected fragments in nucleotides. The expected length (220 nt) of a protected fragment for a mouse analogue of the truncated human MPLK is marked but not observed. Sixty and 20 μg of total RNA from spleen (Spl) or BaF3 cells or yeast tRNA were used for each hybridization with probe A and probe B respectively. No *mpl* mRNA was detected in MEL cells or in FDC-P1 cells (not shown).

polymorphic markers *D4Mit12* and *D4Mit11* are in close proximity to each other on mouse chromosome 4 and to date have not been separated in crosses.

Analysis of alternative splicing

To assess whether the cDNA encoding mpl-tr represents a physiologically transcribed mRNA in mouse tissues we used a ribonuclease (RNase) protection assay (Krieg and Melton, 1987) with probes that distinguish between the full length and the truncated forms. Probe A spans the region deleted in the *mpl-tr* cDNA from the 5' end (Figure 2A) and protects a 250 nucleotide (nt) fragment from mRNA corresponding to mpl and a 190 nt fragment corresponding to mRNA coding for mpl-tr. Probe B is identical to the part of mpl transduced by the myeloproliferative leukemia virus. This probe spans the breakpoint of the deleted region from the 3' end (Figure 2A). A protected fragment f 590 nt was expected for mpl and a 370 nt fragment for mpl-tr. By densitometry of the protected fragments shown in Figure 2B *mpl-tr* accounts for 25% and 40% of the total spleen *mpl* mRNA with probe A and probe B, respectively.

Expression of mpl protein

To study the expression pattern and distribution of mpl protein, we generated rabbit polyclonal anti-mpl antibodies directed against the N-terminal half of the extracellular domain of mpl. Although BaF3 cells express endogenous mRNA corresponding to both forms of mpl, the levels of mpl protein expression are too low for detection by immunoprecipitation or by Western blot (not shown). Therefore, we chose to overexpress mpl in transfected cell lines. To create a full length cDNA for mpl, the 5' end of mpl-tr cDNA was added to the cDNA for mpl. This composite cDNA and the cDNA for mpl-tr were subcloned into the expression vector pcDNA1. Transiently transfected COS cells were metabolically labeled with [^{35}S]methionine, lysed and unique proteins of 78 and 55 kDa were immunoprecipitated from the mpl and mpl-tr transfectants, respectively (Figure 3A). In addition, proteins of 81 and 43 kDa were detected in both mpl and mpl-tr transfectants. The identity of these proteins is at present unknown.

Surprisingly, given the presence of a signal sequence and the absence of the transmembrane and cytoplasmic domains, no mpl-tr protein was found in the tissue culture supernatant (Figure 3A). This might be due to a defective leader peptide, attachment of the mpl-tr protein to the cell membrane through a lipid anchor, intracellular retention of mpl-tr possibly due to the loss of the WSXWS motif, or instability of secreted mpl-tr protein. To show that the mpl signal sequence is functional, we expressed a fusion protein consisting of the entire extracellular domain of mpl fused via the C-terminus to human secreted placental alkaline phosphatase (Figure 4) (Flanagan and Leder, 1990) in transfected COS cells. As shown in Figure 3A, a protein of the expected 135 kDa was readily immunoprecipitated from both cell lysates and supernatant (Figure 3A). As expected, alkaline phosphatase activity was also detected in the supernatant. Again, a protein of 81 kDa appeared in the immunoprecipitate.

To determine whether truncated mpl is associated with the outer cell membrane through a lipid anchor, as has been described for ciliary neurotrophic factor receptor, another member of the cytokine receptor family (Davis *et al.*, 1991), we labeled transiently transfected COS cells with ^{125}I using lactoperoxidase (LPO) (Marchalonis, 1969). Only proteins located on the outside of the cell membrane are labeled by this procedure. We were able to label and immunoprecipitate the full length form of mpl from COS cells transfected with the composite mpl cDNA (Figure 3B), indicating that all elements necessary for correct sorting and transport to the cell surface are present. Mpl-tr, however, was not detected on the surface of COS cells transfected with *mpl-tr* cDNA and appears to be an exclusively intracellular protein. Interestingly, the bands of 81 and 43 kDa observed in the

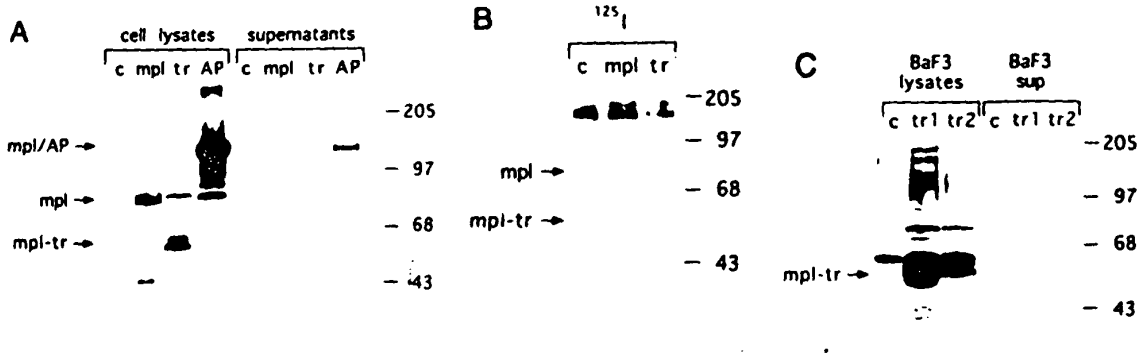


Fig. 3. Immunoprecipitation of *mpl* and *mpl-tr* with anti-mpl antibodies. 'c', indicates mock transfected controls; 'mpl', cells transfected with full length *mpl* cDNA; 'tr', cells transfected with *mpl-tr* cDNA; 'AP', cells transfected with mpl/AP fusion construct. Numbers indicate mol. wt in kDa. (A) Transiently transfected COS cells were metabolically labeled with [^{35}S]methionine. As indicated, cell lysates or culture supernatants were immunoprecipitated with anti-mpl antibodies. (B) ^{125}I/lactoperoxidase cell surface labeling and immunoprecipitation of transiently transfected COS cells. (C) Stably transfected BaF3 cell lines were metabolically labeled with [^{35}S]methionine and cell lysates or culture supernatants were immunoprecipitated with anti-mpl antibodies. The identity of the 63 kDa band present in the cell lysates of transfected BaF3 cells as well as in the control is at present unknown. A similar band was also observed in MEL cells, which do not express *mpl* mRNA (not shown).

[^{35}S]methionine immunoprecipitates were not detected with ^{125}I labeling. Thus, these proteins may be intracellular or they may lack tyrosine residues necessary for LPO labeling.

Since COS cells are monkey cells of non-hematopoietic origin, we hypothesized that co-factors necessary for secretion of mpl-tr might be missing. To test the possibility that mpl-tr might be secreted by hematopoietic cells, we subcloned *mpl-tr* cDNA into a suitable vector (Daley *et al.*, 1990) and tested mpl-tr expression in stably transfected BaF3 cells. Two independent BaF3 clones express mpl-tr in the cell lysate, but did not secrete any detectable mpl protein into the superantant (Figure 3C). Thus, there is no apparent difference between COS cells and BaF3 cells with respect to trafficking of mpl-tr.

hIL4-R/mpl chimera

As is the case for other members of the cytokine receptor superfamily, the cytoplasmic domain of mpl lacks any recognizable enzymatic motif that would hint at a signalling capability. Therefore, it was important to test directly whether mpl is a functional receptor that can transduce a proliferative signal. For this purpose, we designed a chimeric receptor consisting of the cytoplasmic domain of mpl fused to the extracellular and transmembrane domains of the human interleukin-4 receptor (hIL4-R) (Figure 4). This chimera, designated hIL4-R/mpl, was tested for its ability to bind human IL4 and signal when stably transfected into BaF3 cells (Palacios and Steinmetz, 1985). BaF3 is a murine interleukin-3 (IL3) dependent cell line that stops proliferating and undergoes rapid apoptosis when IL3 is withdrawn from the media (Rodriguez-Tarduchy *et al.*, 1990). BaF3 cells stably transfected with the wild type human IL4-R grow normally in human IL4 (D.C.Seldin, in preparation). Thus, the human IL4-R activated by human IL4 can substitute for the IL3-R-mediated proliferative signal. The cytoplasmic domain of the IL4-R is required for signal transduction (Mosley *et al.*, 1989; D.C.Seldin, unpublished results). Therefore, replacing the cytoplasmic domain with an exogenous cytoplasmic domain is a suitable test for activity. In addition, the interaction of human IL4 with its receptor is species specific (Lowenthal *et al.*, 1988); therefore the results are not

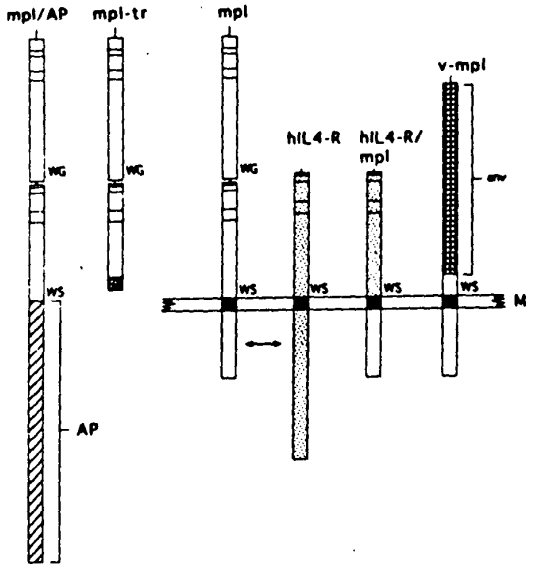


Fig. 4. Schematic representation of mpl and mpl variants used in this study in relation to the oncogenic v-mpl protein. Open boxes represent mpl sequences. Horizontal lines indicate the position of the conserved cysteines. 'WG' and 'WS' indicate the positions of the WGXWS and WSXWS motifs respectively. Mpl/AP is a 135 kDa fusion protein between the extracellular domain of mpl and human placental secreted alkaline phosphatase (hatched). In the hIL4-R/mpl chimera the cytoplasmic domain of the hIL4-R (stippled) was replaced by the corresponding mpl sequence (open box). The transmembrane domain of the chimera is derived from hIL4-R.

complicated by activation of endogenous mouse IL4-R.

The chimeric human IL4-R/mpl was transfected into BaF3 cells and six G418-resistant clones were selected and assayed for expression of the chimeric receptor. Two clones were found to express detectable levels of cell surface binding sites for human IL4, as assayed by binding of a human IL4/AP fusion protein (Figure 5A) (Morrison and Leder, 1992). When IL3 was removed from the culture medium, both cell lines were able to proliferate in the presence of human IL4

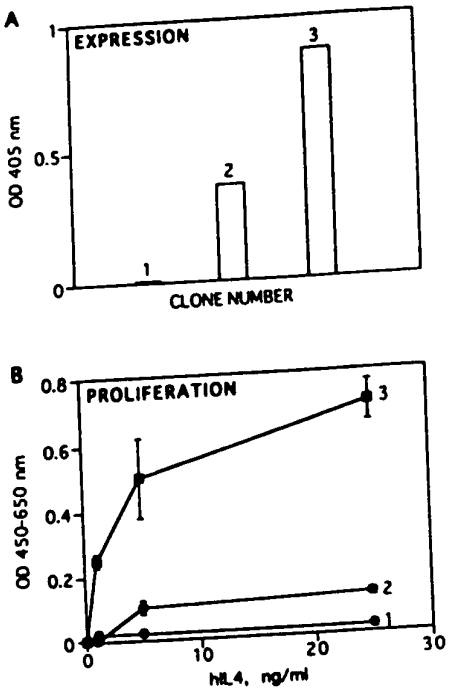


Fig. 5. (A) Expression of the hIL4-R/mpl chimeric receptor. Examples of three G418-resistant clones are shown. The relative expression was assessed by enzymatic assay of bound hIL4/AP fusion protein. The product of *p*-nitrophenyl phosphate cleavage was quantitated by measurement of absorbance at 405 nm. Clone 1 was a non-expressor, and clones 2 and 3 expressed intermediate and higher levels of binding sites for human IL4, as shown. (B) Signalling via the hIL4-R/mpl chimeric receptor. The individual clones were incubated in the presence of increasing doses of human IL4. Proliferation was measured by reduction of XTT, with quantitation of the orange product by absorbance at 450 nm and background subtraction at 650 nm. Error bars indicate the mean and standard deviation for three wells assayed at each concentration.

in a dose-dependent manner (Figure 5B). The maximal response observed with clone 3 is the same as that seen with transfected wild type human IL4-R (not shown). Thus, these results indicate that the cytoplasmic domain of mpl contains all elements necessary to transduce a proliferative signal.

Discussion

Comparison of human and mouse mpl genes

We have isolated cDNAs and a genomic clone for mouse c-*mpl*. The deduced mouse mpl protein is 86% similar and 82% identical to the longer form of human MPL, termed MPLP. This remarkably high degree of conservation extends throughout the entire sequence including the cytoplasmic portion. The extracellular portion can be divided into two subdomains, each having the characteristic four conserved cysteines and a WSXWS or WGXWS motif. A similar duplication is also found in two other members of the cytokine receptor family, namely, the common β-subunit (human β_c; mouse AIC2A and AIC2B) for IL3, IL5 and GM-CSF and the low affinity LIF receptor (LIF-R). This structural feature *per se* does not offer a clue to whether mpl can bind a ligand. AIC2A and LIF-R bind ligand with low affinity, whereas AIC2B and human β_c do not bind ligand at all.

The putative translational start site was present in only one of our cDNAs (*mpl-tr*). However, we were able to deduce the position of the putative initiator ATG from the sequence of a genomic clone for mouse c-*mpl* and constructed a composite full length cDNA by fusing the two longest cDNA clones. This cDNA was expressed in COS cells and anti-mpl antibodies were used to immunoprecipitate a protein of 78 kDa from these cells, slightly larger than the predicted mol. wt of 69 817. Since there are four potential N-glycosylation sites present in the mpl sequence, these may account for the slower mobility on SDS–PAGE.

Chromosomal localization

We have localized c-*mpl* to mouse chromosome 4. On the consensus map of chromosome 4 (Abbot *et al.*, 1992) c-*mpl* is in proximity to the genes for lymphocyte tyrosine kinase (*lck*), L-*myc* and stem cell leukemia (*Scl*). The CA-dinucleotide repeat we have identified and used in the mapping of c-*mpl* should facilitate the inclusion of c-*mpl* as a typable marker in other genetic crosses. Two phenotypic mutants, *Hairpatches* (*Hpt*) (Schultz *et al.*, 1991) and *Repeated epilation* (*Er*) (Guenet *et al.*, 1979), map to this region of chromosome 4. Both exhibit pleiotropic defects; however, a molecular link with c-*mpl* is not obvious from the phenotypes. This region of mouse chromosome 4 is syntenic to human chromosome 1p34 (Nadeau *et al.*, 1992) to which human *MPL* has previously been mapped by *in situ* hybridization (Le Coniat *et al.*, 1989).

Truncated forms of mpl

We identified transcripts encoding a truncated form of mouse mpl (mpl-tr) both by cDNA cloning and by RNase protection. Comparison of sequences of unspliced pre-mRNA and the genomic clone revealed that mpl-tr mRNA is generated by failing to incorporate two exons, resulting in the deletion of the WSXWS motif and the transmembrane domain (Figure 1). We therefore expected this protein to be secreted. However, mpl-tr protein was not detectable in the culture supernatants of transfected COS cells, although an appropriately sized protein was present in the cell lysate.

We tested some of the possible reasons for this discrepancy. The failure of the mpl-tr protein to be exported was not due to a defective signal peptide since the mpl/AP fusion protein was secreted (Figure 3A) and the full length mpl was appropriately directed to the cell membrane (Figure 3B). Mpl-tr was not found on the cell surface by ^{125}I/lactoperoxidase labeling (Figure 3B) and therefore does not appear to be attached to the outside of the cell. BaF3, a hematopoietic cell that normally expresses both forms of mpl at low levels also failed to secrete detectable levels of the transfected truncated form.

Transcripts lacking the transmembrane domain and encoding a potentially soluble form of receptor have been described for most members of the cytokine receptor superfamily (reviewed in Fernandez-Botran, 1991), including the receptors for IL3-Rα, IL4-R, IL5-Rα, IL6-R, IL7-R, IL9-R, G-CSF-Rα, GM-CSF-Rα, LIF-R, GH-R and EPO-R. Alternative splicing was found to be the mechanism in most cases examined. The soluble forms of the IL4-R (Mosley *et al.*, 1989), hIL5-Rα (Tavernier *et al.*, 1991), hIL7-Rα (Goodwin *et al.*, 1990), GM-CSF-R (Raines *et al.*, 1991) and mouse EPO-R (Kuramochi *et al.*, 1990) have been shown to be secreted into the culture supernatant when

overexpressed in COS cells. These soluble receptors were able t bind ligand and had an inhibitory effect by competing for ligand in *in vitro* assays.

The deletion in mpl-tr removes the transmembrane domain as well as the conserved WSXWS motif. The soluble forms of all other members of the cytokine receptor family retain the conserved WSXWS element, with the exception of the soluble human EPO-R (Todok ro *et al.*, 1991). Mutations in the WSXWS motif of the mouse EPO-R resulted in retention of the mutant protein in the endoplasmic reticulum in BaF3 cells (Yoshimura *et al.*, 1992). In contrast, EPO-R mutated in the WSXWS motif was expressed on the cell surface in FDC-P1 cells, but had decreased affinity for ligand and in some cases had lost signalling ability (Quelle *et al.*, 1992). Similarly, deletion of 108 amino acids from the extracellular domain including the WSXWS motif in the G-CSF-R (Fukunaga *et al.*, 1991) and mutations in the WSXWS motif of the IL-2Rβ (Miyazaki *et al.*, 1991) did not interfere with cell surface expression, but in some cases abolished signal transduction. Although the importance of the WSXWS motif in protein trafficking of cytokine receptors remains to be clarified, it is conceivable that the deletion of the WSXWS motif in mpl-tr is responsible for the apparent lack of secretion that we have observed. We cannot exclude that mpl-tr in an appropriate cell type *in vivo* might be efficiently secreted. The secretion process may require additional signals, and a formal possibility is that mpl-tr is secreted, but very unstable and therefore not detectable unless in complex with a soluble cofactor. Alternatively, the truncated form of mpl might have a role within the cell.

Interestingly, a rather different truncated form of MPL has been described in the human (Vigon *et al.*, 1992). This form retains the transmembrane domain, diverges from the full length MPL after nine amino acids of the cytoplasmic domain, and terminates prematurely after 60 amino acids. Three out of a total of eight human *MPL* cDNA clones analyzed were of the truncated type (Vigon *et al.*, 1992), suggesting that this transcript is abundant in humans. We did not find an analogous form in the mouse. RNase protection probe B would have protected a fragment of ~220 nucleotides for an analogous mouse mRNA, but no signal was detected for this form in RNA from spleen or BaF3 cells (Figure 2B). Conversely no analogue corresponding to murine *mpl-tr* has been described in humans (Vigon *et al.*, 1992). Interspecific differences in the expression pattern of alternative forms have been found for other members of the cytokine receptor superfamily, e.g. G-CSF-R (Fukunaga *et al.*, 1990a,b), GM-CSF-Rα (Crossier *et al.*, 1991; Raines *et al.*, 1991) and IL5-Rα (Takaki *et al.*, 1990; Tavernier *et al.*, 1991, 1992). These discrepancies may reflect species differences in hematopoiesis or, alternatively, may simply reflect frequent aberrant splicing events.

Signal transduction by mpl

Since mpl is a member of the cytokine growth factor receptor superfamily, it was important to determine whether it could deliver a growth stimulatory signal. Using a chimeric receptor, we provide evidence that mpl can transduce a proliferative signal. Because of the species specificity of human IL4, the observed effect cannot be due to stimulation of endogenous mouse IL4-R. The maximal response observed with clone 3 is the same as that seen with transfected wild type human IL4-R. The cytoplasmic domain is highly conserved between mouse and human MPL. The conservation (93.3% similarity, 92.5% identity) is even slightly higher than for the extracellular domain (84% similarity, 79.3% identity). Other chimeric constructs will allow us to define the essential elements in the cytoplasmic domain for signalling and to study post-receptor signalling events. At present we cannot predict whether mpl is a single chain receptor capable of binding a ligand or a signalling β-subunit.

The v-*mpl* oncogene is a fusion of the Friend envelope protein to mpl beginning at leucine 448 (Figure 1A). The coding sequences of v-*mpl* and mouse c-*mpl* are identical, but v-*mpl* terminates prematurely omitting the two C-terminal amino acids. The truncated Friend envelope protein gp55 has been shown to associate with the EPO-R on erythroid precursor cells and to constitutively activate the EPO-R (Li *et al.*, 1990). The region required for this activation has been mapped to the transmembrane domain of gp55 (Chung *et al.*, 1989; Showers *et al.*, 1993). Since in v-*mpl* the region encoding the viral transmembrane domain has been replaced by c-*mpl* sequences encoding the mpl transmembrane domain (Figure 4), the transforming mechanisms of gp55 and v-*mpl* cannot be the same. Our data show that the cytoplasmic domain of mpl is competent for signalling and perhaps it is constitutively activated and responsible for cell transformation in the v-*mpl* fusion. The broad range of hematopoietic lineages transformed by v-*mpl* contrasts with the erythroid specificity of gp55. This may reflect the ability of mpl to transmit a proliferative signal in a broad range of hematopoietic lineages or to act at an early myeloid progenitor stage.

Materials and methods

Cloning of mpl

Oligonucleotides starting at position 301 of the published v-*mpl* sequence (Souyri *et al.*, 1990) (5'-CTAGAGCTGCGCCCCCGAGC-3') and antisense oligonucleotides at position 890 of v-*mpl* (5'-ATAGGTCTGCAGTAG-CATGG-3') were used to amplify a 590 bp *mpl* fragment from 1st strand spleen cDNA by PCR. The PCR reactions were carried out in a volume of 100 μl containing 1 μg of each oligonucleotide at an annealing temperature of 55°C. This cDNA was subcloned into pBluescript II (Stratagene) and sequenced by the dideoxy termination method (Sanger *et al.*, 1977) with a modified T7 polymerase (USB) using oligonucleotides covering the sequence. A directional cDNA library was constructed from spleen mRNA of FVB/N mice in Lambda-Zap vector (Stratagene). After adapter ligation the cDNA was size selected by agarose gel electrophoresis and cDNA 2 kb or larger was ligated to the λ-arms. The unamplified library was screened with the 590 bp v-*mpl* fragment labeled with [^{32}P]dCTP. Positive clones were plaque purified, excised with helper phage and sequenced.

A genomic library from the PCC4 teratocarcinoma cell line in Lambda FIX II vector (Stratagene) was screened with a [^{32}P]dCTP labeled *Eco*RI–*Sal*I fragment representing the 5' end of *mpl* clone 8. Positive clones were subcloned into pBluescript II.

Chromosomal localization

An oligonucleotide at position −1047 (5'-CATACTCCTCTTTTCAAC-CA-3') and an antisense oligonucleotide at position −832 (5'-CTGGTT-GTCTGCTTCATTTC-3') flanking a CA-dinucleotide repeat 1 kb upstream of the mpl translational start site amplify a 205 bp product from C57BL/6J DNA, a 185 bp product from DBA/2J DNA and a 195 bp product from *M.spretus* DNA. These polymorphisms were used to type the strain distribution pattern (SDP) of c-*mpl* in the BXD RI strains and in an interspecific backcross of (C57BL/6 × *M.spretus*) × *M.spretus* using DNA obtained from the Jackson Laboratory. PCR reactions were carried out in a volume of 40 μl containing 100 ng of each oligonucleotide primer and 100 ng of genomic template DNA, at an annealing temperature of 55°C with 1.5 mM $MgCl_2$. The polymorphic products were resolved by electrophoresis using both a 1.4% Tris-borate agarose gel and 8% PAGE. Analysis of the SDP was carried out using the R! manager version 2.3 computer program (Manly

and Elliott, 1991), with the following addendum: we have typed BXD strains 1, 22, 30, 31 and 32, which were previously undefined for the simple sequence length repeat polymorphic marker D4Mit12 (Dietrich *et al.*, 1992).

RNase protection assay

RNase protection probe A was generated by PCR using an oligonucleotide (5'-AAGGTGCCGTTCACAGCTAC-3') starting at position 1088 and an antisense oligonucleotide (5'-CCTCGGGGGCGCAGCTCTAG-3') starting at position 1337 from *mpl 8* cDNA as a template and subcloned into pBluescript. The full length v-*mpl* sequence (590 bp) in pBluescript was used as probe B. RNA from mouse tissues or cell lines was prepared by the guanidium thiocyanate/CsCl method (Chirgwin *et al.*, 1979). RNase protection was performed as described (Krieg and Melton, 1987). Sixty and 20 μg of total RNA were used for each sample with probe A and probe B, respectively. Quantitation of bands observed on autoradiograms was performed on a Molecular Dynamics Computing Densitometer.

Construction of a full length mpl cDNA, mpl/AP and hIL4-R/mpl chimera

To generate a full length cDNA for mpl the 5' end of clone *mpl 8* was replaced with the 5' end of the longer clone *mpl 4* coding for mpl-tr, taking advantage of a unique *Sna*I site at position 710. *Mpl 8* in pBluescript II was cut with *Eco*RI and *Sna*I and ligated to an *Eco*RI–*Sna*I fragment from *mpl 4*. Clones with the correct replacement were selected by restriction endonuclease analysis.

A cDNA coding for an mpl/AP fusion protein was constructed in two steps. First a *Hind*III–*Xho*I fragment from the APtag vector (Flanagan and Leder, 1990) containing the alkaline phosphatase region was subcloned into a pcDNA1 vector (InVitrogen) cut with *Hind*III–*Xho*I. The entire extracellular domain of mpl was amplified by PCR from the full length *mpl* cDNA with a sense primer (5'-GAAATTAACCCTCACTAAG-3'), corresponding to the T_3 recognition sequence in pBluescript II and an antisense primer (5'-TTAGATCTCCAAGCAGTCTCGGAGCCCG-3'), starting at position 1446, that introduces a unique *Bgl*II site. The PCR product was purified on agarose gel, cut with *Hind*III and *Bgl*II and ligated into the modified pcDNA1 vector that had been cut with *Hind*III and *Bgl*II.

Recombinant PCR was used to generate a chimeric receptor containing the extracellular and transmembrane domains of the human IL4 receptor (hIL4-R) and the cytoplasmic domain of mpl. A 21 nucleotide sense primer (oligo 1: 5'-CTGACCTGGAGCAACCCGTAT-3') beginning at nucleotide 596 of the hIL4-R, 5' to a unique *Hin*cII site and a 30 nucleotide antisense primer (oligo 2: 5'-AGGAAATTGCCACTTGGTGATGCTGACATA-3') which encoded the last five amino acids of the hIL4-R transmembrane domain and the first five amino acids of the mpl cytoplasmic domain were used in 30 cycles of PCR with a plasmid containing the full length hIL4-R cDNA as a template. The complementary 30 nucleotide sense primer (oligo 3: 5'-TATGTCAGCATCACCAAGTGGCAATTTCCTGC-3') and an antisense primer to the last 15 nucleotides of the v-*mpl* cDNA followed by a stop codon, *Bam*HI and *Eco*RI recognition sequences, and a trinucleotide spacer (oligo 4: 5'-CTTGAATTCGGATCCCTGCTACCAATAGCTTAG-TGGT-3') were used in a parallel PCR with the *mpl* cDNA as template. 0.5% of each reaction product was then mixed, denatured, annealed and extended with Taq polymerase to form the chimeric receptor. This chimeric molecule was amplified for 30 cycles of PCR using oligos 1 and 4 as primers. The product was gel purified using GeneClean (Bio 101), cut with *Hin*cII and *Eco*RI, and cloned into a PGEM-7zf+ plasmid (Promega) containing the wild type hIL4-R sequence 5' to the *Hin*cII site. The fidelity of this construct was confirmed by sequencing. From there, the chimeric receptor was subcloned into the expression vector pGD containing TK-neo and a Moloney LTR promoter (Daley *et al.*, 1990) and termed pGD-hIL4-R/mpl.

Generation of anti-mpl antibodies

An oligonucleotide (5'-CGTAGATCTGGCACAGAGCCCCTGAAC-TGC-3') at position 100 and an antisense oligonucleotide (5'-CACAGA-TCTTCCTGGAAGATCCACAGTCAC-3') at position 822 amplify a 722 bp fragment from mpl clone 8 as a template. Both primers contain a unique *Bgl*II site. This fragment was gel isolated, cut with *Bgl*II and subcloned preserving the reading frame into the *Bam*HI site of the expression vector pQE-8 (Qiagen) which introduces a C-terminal six histidine tag resulting in the C-terminal amino acid sequence: GSETAWRSHHHHHH. Clones positive for expression of mpl after induction with IPTG were identified by SDS–PAGE. The 29 kDa mpl/6His fusion protein was affinity purified on a Ni^{2+} column and further purified by preparative SDS–PAGE. The protein band was cut out from the gel and 100 μg was mixed with complete Freund's adjuvant and injected subcutaneously into rabbits. The boost was performed after 4 weeks with 50 μg of the same immunogen in incomplete Freund's adjuvant. Serum was collected 10 days after the boost and used at a 1:200 dilution for immunoprecipitations and Western blots.

Cell transfections and immunoprecipitations

COS cell transfections were performed by the DEAE–Dextran method (Seed and Aruffo, 1987). Transfected COS cells were radioactively labeled 48–72 h after transfection. Metabolic labeling was carried out in methionine-free DMEM media supplemented with 10% dialyzed serum and 0.25 mCi/ml of [^{35}S]methionine for 6 h at 37°C. Cell surface labeling with ^{125}I and lactoperoxidase (Marchalonis, 1969) was carried out *in situ* on COS cells growing attached to the Petri dish in PBS with 2 mCi/ml ^{125}I. Labeled cells were washed twice in PBS and lysed for 10 min on ice in 1 × TBS (150 mM NaCl, 50 mM Tris pH 8) with 1% Triton X-100 and protease inhibitors. The lysates were spun for 30 min at 10 000 *g*. Supernatants were adjusted to 0.2% SDS, 0.5% NP40 and 0.5% deoxycholate final concentration and incubated with antibodies at 1:200 dilution for 12 h at 4°C. Protein A–Sepharose beads (Pharmacia) were added, incubated for 2 h and washed four times in RIPA buffer (0.1% SDS, 0.5% NP40 and 0.5% deoxycholate in TBS) and twice with TBS. The immune complexes were eluted with 0.1 M glycine pH 3 for 1 min and neutralized with 1 M Tris base and 4 × SDS–PAGE loading buffer.

BaF3 cells were electroporated with 40 μg of pGD-mpl-tr DNA or pGD-hIL4-R/mpl DNA at 250 V/960 μF in PBS and plated in serial dilutions. Clones were selected in 0.6 mg/ml G418 beginning at 24 h. Metabolic labeling of BaF3 clones transfected with pGD-mpl-tr and immunoprecipitations were performed as described above.

Expression and analysis of the chimeric hIL4-R/mpl receptor in BaF3 cells

Stable BaF3 clones transfected with pGD-hIL4-R/mpl were selected and assayed for expression of the chimeric receptor by binding of a human IL4/human placental alkaline phosphatase fusion protein (Morrison and Leder, 1992). The binding was quantitated by measuring alkaline phosphatase activity (Flanagan and Leder, 1990). The function of the hIL4-R/mpl receptors was determined by washing the cells out of IL3-containing medium and plating them in 96 well plates at 10^4 cells per well in 100 μl of medium containing increasing concentrations of conditioned medium from transiently transfected COS cells secreting human IL4. The human IL4 concentration was determined by comparison with a sample from the National Institute for Biological Standards and Control. After 3 days of stimulation, 50 μl of a 1 mg/ml stock solution of XTT, a colorimetric dye [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide] with 5 mM PMS (phenazine methosulfonate), an electron coupling agent, was added to each well (Scudiero *et al.*, 1988). The product of XTT reduction by viable cells, reflecting the number of cells per well, was measured at 4 h on an automated plate reader (Molecular Devices).

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We would like to thank Briggs Morrison for the cDNA for hIL4/AP. We would like to thank Joe Nadeau, Ed Birkenmeier and Muriel Davisson for the access to the interspecific (C57BL/6 × *M.spretus*) × *M.spretus* backcross. R.C.S. was supported in part by the Schweizerische Stiftung für medizinisch-biologische Stipendien, D.C.S. was supported in part by National Institutes of Health award #5K08HL02686-02.

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Received on March 10, 1993

Note added in proof

The cDNA sequence and the genomic sequence data reported here have been deposited in the EMBL and GenBank Nucleotide Sequence databases under the accession numbers Z22649 and Z22657 respectively.

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    21jan00 11:23:55 User217743 Session D497.1
       $0.00   0.198 DialUnits FileHomeBase
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    (c) 1999 The Dialog Corporation plc

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   $0.01 TYMNET
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DIALINDEX(R)
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*** DIALINDEX search results display in an abbreviated
*** *** format unless you enter the SET DETAIL ON
command. *** ? set files biochem

>>>       162 is unauthorized
>>>1 of the specified files is not available
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  (To see banners, use SHOW FILES command)
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  6: NTIS_64-2000/Feb W1
 34: SciSearch(R) Cited Ref Sci_1990-2000/Jan W3
 40: Enviroline(R)_1975-1999/Oct
 41: POLLUTION ABS_1970-2000/JAN
 50: CAB ABSTRACTS_1972-1999/DEC
 65: Inside Conferences_1993-1999/Jun W3
 68: Env.Bib._1974-2000/Nov
 71: ELSEVIER BIOBASE_1994-2000/Nov W3
 73: EMBASE_1974-2000/Jan W1
 76: LIFE SCIENCES COLLECTION_1982-2000/NOV
 77: CONFERENCE PAPERS INDEX_1973-2000/JAN
 94: JICST-EPlus_1985-2000/Oct W2
 98: General Sci Abs/Full-Text_1984-1999/Oct
103: ENERGY SCITEC_1974-2000/DEC B2
143: Biol. & Agric. Index_1983-2000/Dec
144: PASCAL_1973-2000/DEC
155: MEDLINE(R)_1966-2000/Mar W2
156: TOXLINE(R)_1965-1999/DEC
172: EMBASE Alert_2000/Jan W1
305: Analytical Abstracts_1980-2000/Jan W3
370: Science_1996-1999/Jul W3
399: CA SEARCH(R)_1967-2000/UD=13204
434: SciSearch(R) Cited Ref Sci_1974-1989/Dec

? s mpl and (antibody or antibodies) and agonist?


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  s mpl and (antibody or antibodies) and agonist?

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PASCAL_1973-2000/DEC
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SEARCH(R)_1967-2000/UD=13204
N7          2   77: CONFERENCE PAPERS
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(c) 2000 Inst for Sci Info

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2/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09648937 98402345
An %agonist% murine monoclonal %antibody% to the human c-%Mpl% receptor stimulates megakaryocytopoiesis.
Deng B; Banu N; Malloy B; Hass P; Wang JF; Cavacini L; Eaton D; Avraham H Divisions of Experimental Medicine and Hematology/Oncology, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, Boston, MA and Genentech Inc, South San Francisco, CA, USA.
Blood (UNITED STATES) Sep 15 1998, 92 (6) p1981-8, ISSN 0006-4971 Journal Code: ABG
Contract/Grant No.: RO1 51456
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Thrombopoietin (TPO) is a hematopoietic growth factor that stimulates megakaryocytopoiesis and platelet production in vivo and promotes the development of identifiable megakaryocytes in vitro. We have developed a murine monoclonal %antibody% , BAH-1, raised against human megakaryocytic cells, which specifically recognizes the c-%Mpl% receptor and shows %agonist% activity by stimulating megakaryocytopoiesis in vitro. BAH-1 %antibody% specifically binds to platelets and to recombinant c-%Mpl% with high affinity. Similar to TPO, BAH-1 alone supported the formation of colony-forming unit-megakaryocyte (CFU-MK) colonies. The combination of BAH-1 plus interleukin-3 or of BAH-1 plus human TPO significantly increased the number of human CFU-MK colonies. In addition, BAH-1 monoclonal %antibody% stimulated the proliferation and maturation of primary bone marrow megakaryocytes in a dynamic heterogeneous liquid culture system. Individual large megakaryocytes as well as small megakaryocytic cells were observed in cultures of CD34(+) CD41(+) cells in the presence of BAH-1 %antibodies%. Similar to TPO, BAH-1 %antibody% induced a significant response of murine immature megakaryocytes as observed by an increase in the detectable numbers of acetylcholinesterase-positive megakaryocytes. No effects of BAH-1 %antibody% were observed on colony-forming unit-granulocyte-macrophage, burst-forming unit-erythroid, or colony-forming unit-erythroid colonies. In vivo studies showed that BAH-1, alone or in combination with TPO, expands the numbers of megakaryocytic progenitor cells in myelosuppressed mice. This %antibody% should prove useful in understanding the structure-function aspects of the c-%Mpl% receptor as well as in evaluating the effects of the sustained activation of this receptor in preclinical models of severe thrombocytopenia.

2/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09454437 98183393
Autocrine inhibition of leptin production by tumor necrosis factor-alpha (TNF-alpha) through TNF-alpha type-I receptor in vitro.

Yamaguchi M; Murakami T; Tomimatsu T; Nishio Y; Mitsuda N; Kanzaki T; Kurachi H; Shima K; Aono T; Murata Y

Department of Obstetrics and Gynecology, Osaka University Medical School, Suita, Japan.

Biochem Biophys Res Commun (UNITED STATES) Mar 6 1998, 244 (1) p30-4, ISSN 0006-291X Journal Code: 9Y8

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The aim of this study was to find factors which regulate m-leptin secretion during pregnancy. Mouse parametrial adipocytes from day 13 of pregnancy were cultured with or without mouse placental lactogen (%mPL%)-I, %mPL% -II, or mouse tumor necrosis factor-alpha (mTNF-alpha) and mouse-leptin (m-leptin) concentration in the medium was assessed by RIA. Up to four days of %mPL%-I or %mPL%-II treatment did not affect m-leptin secretion. However, mTNF-alpha, which is produced by adipocytes, significantly inhibited m-leptin secretion in a dose- and time-dependent manner. %Antibody% to mTNF-alpha completely blocked the inhibitory effect of mTNF-alpha on m-leptin secretion. mTNF-alpha significantly inhibited the expression of m-leptin messenger RNA. %Agonistic% polyclonal %antibody% directed against the mTNF-type-I receptor (mTNF-RI) significantly inhibited m-leptin secretion, but the anti-mTNF-RII %antibody% did not change m-leptin secretion. Moreover, human TNF-alpha (h-TNF-alpha) also inhibited human-leptin (h-leptin) secretion by cultured human adipocytes collected from the subcutaneous fat of pregnant women. These results suggest that TNF-alpha, which is secreted by adipocytes, inhibits m-leptin secretion through mTNF-RI and suggest the presence of an autocrine or paracrine regulation of leptin secretion in human and mouse adipose tissue in vivo.

2/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09209147 97399692

Direct demonstration of MuSK involvement in acetylcholine receptor clustering through identification of %agonist% ScFv [see comments] Xie MH; Yuan J; Adams C; Gurney A

Department of Molecular Biology, Genentech, Inc., San Francisco, CA 94080, USA.

Nat Biotechnol (UNITED STATES) Aug 1997, 15 (8) p768-71, ISSN 1087-0156 Journal Code: CQ3

Comment in Nat Biotechnol 1997 Aug;15(8):721-2

Languages: ENGLISH

Document type: JOURNAL ARTICLE

MuSK is a tyrosine kinase localized to the postsynaptic surface of the neuromuscular junction. We have searched for modulators of MuSK function using a library of human single chain variable region %antibodies% (scFv) that can be displayed on M13 phage or expressed as soluble protein. A panel of 21 independent MuSK-specific scFv, identified in a screen for binding to MuSK-Fc immunoadhesin, were examined for ability to induce proliferation in a factor dependent cell line (Ba/F3) through a chimeric receptor, MuSK- %Mpl% . Four of the scFv induced a proliferative response, suggesting an ability to induce dimerization of MuSK. These scFv were also able to induce tyrosine phosphorylation of full-length MuSK and retained this ability when re-engineered to be expressed as authentic (and dimeric) human IgG molecules. Addition of %agonist% scFv to a cultured myotube cell line induced AChR clustering and tyrosine phosphorylation. These results provide direct evidence that MuSK activation is capable of triggering a key event in neuromuscular junction formation and further demonstrate that large libraries of phage-displayed scFv provide a robust method for generating highly specific %agonist% agents.

2/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09008395 97220127

Thrombopoietin is synergistic with other hematopoietic growth factors and physiologic platelet %agonists% for platelet activation in vitro. Wun T; Paglieroni T; Hammond WP; Kaushansky K; Foster DC Department of Internal Medicine, University of California at Davis School of Medicine, California, USA.

Am J Hematol (UNITED STATES) Mar 1997, 54 (3) p225-32, ISSN 0361-8609 Journal Code: 3H4

Contract/Grant No.: R29HL55181, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Thrombopoietin (TPO) is the primary physiologic regulator of platelet production. The effect of TPO on platelet function, both alone and in combination with other hematopoietic growth factors, adenosine diphosphate (ADP), and epinephrine, was investigated using fluorescent-labeled %antibodies% to the activation-dependent antigen CD62 (P-selectin) and flow cytometry. TPO stimulated CD62 expression on normal human platelets, and this expression was completely inhibited by the soluble extracellular domain of the TPO receptor, %MPL% . The growth factors granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO), but not interleukin-3 (IL-3) or stem-cell factor (SCF), also stimulated platelet activation. The combination of EPO, SCF, ADP, and epinephrine with TPO were synergistic for platelet CD62 expression. These

data further support a role for TPO in modulating platelet function.

2/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

07976066 94338384
Tumor necrosis factor-alpha (TNF-alpha) inhibits expression of mouse placental lactogen-II through TNF-alpha type-I but not type-II receptor. Yamaguchi M; Kurachi H; Tadokoro C; Sakata M; Yoshimoto Y; Masumoto N; Tasaka K; Miyake A
Department of Obstetrics and Gynecology, Osaka University Medical School, Japan.
Biochem Biophys Res Commun (UNITED STATES) Aug 15 1994, 202 (3) p1599-605, ISSN 0006-291X Journal Code: 9Y8
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The aim of this study was to determine whether TNF-alpha inhibits %mPL% -II secretion through TNF-RI or TNF-RII, and to investigate the gestational profile of TNF-RI and TNF-RII gene expression. The mouse trophoblast cells from day 12 pregnancy were cultured with or without %agonistic% polyclonal %antibodies% directed against the individual TNF-alpha receptors, and %mPL% -II secretion in the medium was assessed by RIA. Anti-TNF-RI %antibody% significantly inhibited the %mPL% -II secretion in a dose- and time-dependent manner, but anti-TNF-RII %antibody% did not. Moreover, the TNF-RII %antibody% did not influence the inhibitory effect of the TNF-RI %antibody% on %mPL%-II secretion. TNF-RI %antibody% inhibited the %mPL%-II gene expression by Northern blot analysis. Amount of the gene expression of TNF-RI in the second half of pregnancy was higher than those in the first half of pregnancy both in vitro and in vivo, although the gene expression of TNF-RII was not detectable by Northern blot analysis using poly(A)+RNA. These results suggest that TNF-RI is a main receptor for TNF-alpha in mouse placenta and that TNF-alpha may have an important role in regulating %mPL% -II secretion after midpregnancy.

2/3,AB/6 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

08007509 Genuine Article#: 235WF Number of References: 41 Title: Small molecule cytokine mimetics
Author(s): Whitty A (REPRINT) ; Borysenko CW
Corporate Source: BIOGEN INC,DEPT PROT ENGN, 14 CAMBRIDGE CTR/CAMBRIDGE//MA/02142 (REPRINT)
Journal: CHEMISTRY & BIOLOGY, 1999, V6, N4 (APR), PR107-R118 ISSN: 1074-5521 Publication date: 19990400
Publisher: CURRENT BIOLOGY LTD, 34-42 CLEVELAND STREET, LONDON W1P 6LE, ENGLAND
Language: English Document Type: REVIEW
Abstract: number of reports describe small peptides, and even bona fide small organic molecules, that activate homodimeric cytokine receptors and show cytokine-like activity in vitro and in vivo. These cases can be examined in light of the mechanistic and thermodynamic principles that govern cytokine-receptor activation.

2/3,AB/7 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

06775961 Genuine Article#: ZR105 Number of References: 19 Title: Surrogate thrombopoietin
Author(s): Abe M (REPRINT) ; Yano S; Sakaba N; Kitamura K; Urasaki T; Nakada S; Kawasaki H; Morimoto C; Masuho Y
Corporate Source: YAMANOUCHI PHARMACEUT CO LTD,INST DRUG DISCOVERY RES, 21 MIYUKIGAOKA/TSUKUBA/IBARAKI 305/JAPAN/ (REPRINT); UNIV TOKYO,INST MED SCI, MINATO KU/TOKYO 108//JAPAN/
Journal: IMMUNOLOGY LETTERS, 1998, V61, N2-3 (APR), P73-78 ISSN: 0165-2478 Publication date: 19980400
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS Language: English
Document Type: ARTICLE
Abstract: The extracellular domain of human c-%Mpl%, the receptor for thrombopoietin (TPO), was expressed as a chimeric protein with the interleukin-2 receptor alpha chain on the surface of murine B cell-line B300-19. BALB/c mice were immunized with cells expressing the chimeric protein. The IgG purified from the resulting immune serum immunoprecipitated human c-%Mpl%. The immune IgG supported proliferation of both stable transfectant Ba/F3 cells expressing whole c-%Mpl% molecules (c-%Mpl%-Ba/F3 No. 9) and UT7/TPO cells bearing naturally occuring c-%Mpl%, whereas it did not support the growth of the untransfected parental Ba/F3 cells. Cell growth was induced using 3 to 100 mu g/ml of immune IgG in a dose-dependent manner, but this induction was decreased at doses higher than 100 mu g/ml. Non-immune IgG did not affect cell growth of c-%Mpl%-Ba/F3 No. 9 cells. Although the Fab fragment of immune IgG also immunoprecipitated c-%Mpl%, it did not support cell growth at concentrations as high as 180 mu g/ml, implying that the bivalent binding of receptors by %antibodies% is

essential for cell proliferation. These results suggest that %antibodies% against human c-%Mpl% stimulate the proliferation and differentiation of megakaryocytes by their bivalent binding to receptors like TPO. (C) 1998 Elsevier Science B.V. All rights reserved.

2/3,AB/8 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

06438656 Genuine Article#: YT425 Number of References: 60 Title: A structural model of the human thrombopoietin receptor complex Author(s): Deane CM; Kroemer RT; Richards WG (REPRINT)
Corporate Source: UNIV OXFORD,PHYS & THEORET CHEM LAB, S PARKS RD/OXFORD OX1 3QZ//ENGLAND/ (REPRINT); UNIV OXFORD,PHYS & THEORET CHEM LAB/OXFORD OX1 3QZ//ENGLAND/
Journal: JOURNAL OF MOLECULAR GRAPHICS & MODELLING, 1997, V15, N3 (JUN), P 170-&
ISSN: 1093-3263 Publication date: 19970600
Publisher: ELSEVIER SCIENCE INC, 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010
Language: English Document Type: ARTICLE
Abstract: Thrombopoietin (TPO) is a glycoprotein hormone that regulates red blood cell production. Presented here is a modeling study of the extracellular region of the human thrombopoietin receptor complex, in particular the TPO-receptor interface. The models were developed from structural homology to other cytokines and their receptors. Experimental evidence suggests that the receptors. Experimental evidence suggests that the receptor is homodimeric and it was modeled accordingly. Key interactions are shown that correlate with previous cytokine receptor complexes, and the pattern of cysteine bonding (Cys7-Cys151 and Cys29-Cys85) agrees with that experimentally determined for thrombopoietin. These models pave the way for possible mutagenesis experimentation and the design of (ant)%agonists%. (C) 1997 by Elsevier Science Inc.

2/3,AB/9 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06296626 Genuine Article#: YG424 Number of References: 0 Title: Development of potent %agonist% %antibodies% to c-%Mpl% from a human scFv phage display library
Author(s): Adams C; Hass P; Schroeder K; Jung C; Malloy B; Suggett S; Sims P; Nagel M; Fendly B; Eaton D
Corporate Source: GENENTECH INC,/S SAN FRANCISCO//CA/94080 Journal: BLOOD, 1997, V90, N10,1,1 (NOV 15), P236-236
ISSN: 0006-4971 Publication date: 19971115
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399
Language: English Document Type: MEETING ABSTRACT

2/3,AB/10 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05999167 Genuine Article#: XN024 Number of References: 39 Title: Sense and antisense RNA for the membrane associated 40 kDa subunit M40 of the insect V-ATPase
Author(s): Merzendorfer H; Harvey WR; Wieczorek H (REPRINT) Corporate Source: UNIV MUNICH,INST ZOOL, LUISENSTR 14/D-80333 MUNICH//GERMANY/ (REPRINT); UNIV MUNICH,INST ZOOL/D-80333 MUNICH//GERMANY/
Journal: FEBS LETTERS, 1997, V411, N2-3 (JUL 14), P239-244 ISSN: 0014-5793 Publication date: 19970714
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS Language: English Document Type: ARTICLE
Abstract: For the first time a cDNA encoding the membrane associated subunit M40 of an invertebrate V-ATPase has been isolated and sequenced, based on a cDNA library from larval midgut of the tobacco hornworm, Manduca sexta, Immunoblotting with monospecific %antibodies% raised against the recombinant M40 polypeptide demonstrated that it is a subunit of the insect plasma membrane V-ATPase, Since M40 subunits had been identified only in endosomal V-ATPases till now, this result indicates that they are constitutive members of all, endomembrane and plasma membrane V-ATPases,

A phagemid clone representing a polyadenylated antisense transcript was also isolated and sequenced, Using RT-PCR, endogenous antisense RNA was detected in poly(A) RNA isolated from the larval midgut, Since Southern blots indicated a single gene locus, both the antisense RNA as well as the sense mRNA encoding subunit M40 seem to originate from the same gene. (C) 1997 Federation of European Biochemical Societies.

2/3,AB/11 (Item 6 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05319029 Genuine Article#: VP736 Number of References: 44 Title: ADMINISTRATION OF PEGYLATED RECOMBINANT HUMAN MEGAKARYOCYTE GROWTH AND DEVELOPMENT FACTOR TO HUMANS

STIMULATES THE PRODUCTION OF FUNCTIONAL PLATELETS THAT SHOW NO EVIDENCE OF IN-VIVO ACTIVATION Author(s): OMALLEY CJ; RASKO JEJ; BASSER RL; MCGRATH KM; CEBON J; GRIGG AP; HOPKINS W; COHEN B; OBYRNE J; GREEN MD; FOX RM; BERNDT MC; BEGLEY CG Corporate Source: UNIV MELBOURNE,ROYAL MELBOURNE HOSP,WALTER & ELIZA HALL INST MED RES/MELBOURNE/VIC 3050/AUSTRALIA/; UNIV MELBOURNE,ROYAL MELBOURNE HOSP,WALTER & ELIZA HALL INST MED RES/MELBOURNE/VIC 3050/AUSTRALIA/; CTR DEV CANC THERAPEUT/MELBOURNE/VIC/AUSTRALIA/; LUDWIG INST CANC RES,MELBOURNE TUMOUR BIOL BRANCH/MELBOURNE/VIC/AUSTRALIA/; ROYAL MELBOURNE HOSP,ROTARY BONE MARROW RES LABS/MELBOURNE/VIC/AUSTRALIA/; WALTER & ELIZA HALL INST MED RES/PARKVILLE/VIC/AUSTRALIA/; AMGEN AUSTRALIA/KEW/VIC/AUSTRALIA/; AMGEN CORP/THOUSAND OAKS//CA/91320
Journal: BLOOD, 1996, V88, N9 (NOV 1), P3288-3298 ISSN: 0006-4971
Language: ENGLISH Document Type: ARTICLE
Abstract: This report describes the effect of pegylated recombinant human megakaryocyte growth and development factor (PEG-rHUMGDF) on platelet production and platelet function in humans. Subjects with advanced solid tumors received PEG-rHuMGDF daily for up to 10 days. There was no increase in circulating platelet count at doses of 0.03 or 0.1 mu g/kg/d by day 12 of study. At doses of 0.3 and 1.0 mu g/kg/d there was a threefold median increase (maximum 10-fold) in platelet count by day 16. The platelets produced in vivo in response to PEG-rHuMGDF showed unchanged aggregation and adenosine triphosphate (ATP)-release responses in in vitro assays. Tests included aggregation and release of ATP in response to adenosine diphosphate (ADP) (10, 5, 2.5, and 1.25 mu mol/L). collagen (2 mu g/mL), thrombin-receptor %agonist% peptide (TRAP, 10 mu mol/L) and ristocetin (1.5 mg/mL). Administration of aspirin to an individual with a platelet count of 1,771x10(9)/L resulted in the typical aspirin-induced ablation of the normal aggregation and ATP-release response to stimulation with arachidonic acid (0.5 mg/mL), collagen, and ADP (2.5 and 1.25 mu mol/L). There was no change in the expression of the platelet-surface activation marker CD62P (P-selectin) nor induction of the fibrinogen binding site on glycoprotein IIb/IIIa as reported by the monoclonal %antibody%, D3GP3. An elevation of reticulated platelets was evident after 3 days of treatment with PEG-rHuMGDF and preceded the increase in circulating platelet count by 5 to 8 days; this reflected the production of new platelets in response to PEG-rHuMGDF. At later time points, the mean platelet volume (MPV) decreased in a manner inversely proportional to the platelet count. Levels of plasma glycocalicin, a measure of platelet turnover, rose 3 days after the initial increase in the peripheral platelet count. The level of plasma glycocalicin was proportional to the total platelet mass, suggesting that platelets generated in response to PEG-rHuMGDF were not more actively destroyed. Thus, the administration of PEG-rHuMGDF, to humans, increased the circulating platelet count and resulted in fully functional platelets, which showed no detectable increase in reactivity nor alteration in activation status. (C) 1996 by The American Society of Hematology.

2/3,AB/12 (Item 7 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05143396 Genuine Article#: VC092 Number of References: 0 Title: AN %AGONIST% MURINE MONOCLONAL-%ANTIBODY% TO HUMAN C-%MPL% RECEPTOR STIMULATES IN-VITRO MEGAKARYOCYTOPOIESIS
Author(s): DENG B; WANG JF; BANU N; CAVACINI L; GROOPMAN JE; AVRAHAM H Corporate Source: HARVARD UNIV,NEW ENGLAND DEACONESS HOSP,SCH MED,DIV HEMATOL ONCOL/BOSTON//MA/02215
Journal: EXPERIMENTAL HEMATOLOGY, 1996, V24, N9 (AUG), P260 ISSN: 0301-472X
Language: ENGLISH Document Type: MEETING ABSTRACT

2/3,AB/13 (Item 8 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

04966625 Genuine Article#: UW488 Number of References: 70 Title: DOSE-RESPONSE EFFECTS OF PEGYLATED HUMAN MEGAKARYOCYTE GROWTH AND DEVELOPMENT FACTOR ON PLATELET PRODUCTION AND FUNCTION IN NONHUMAN-PRIMATES
Author(s): HARKER LA; MARZEC UM; HUNT P; KELLY AB; TOMER A; CHEUNG E; HANSON SR; STEAD RB Corporate Source: EMORY UNIV,SCH MED,DIV HEMATOL & ONCOL,YERKES REG PRIMATE RES CTR,1639 PIERCE DR,ROOM 1003/ATLANTA//GA/30322; AMGEN INC/THOUSAND OAKS//CA/91320
Journal: BLOOD, 1996, V88, N2 (JUL 15), P511-521 ISSN: 0006-4971
Language: ENGLISH Document Type: ARTICLE
Abstract: Thrombopoietin (TPO) is the physiologic %Mpl%-ligand regulating platelet production. Pegylated human recombinant megakaryocyte growth and development factor (PEG-rHuMGDF), a truncated

polypeptide %Mpl% -ligand derivitized with poly-(ethylene glycol), induces megakaryocyte endoreduplication and proliferation in vitro and in vivo. In the present study, the dose-response effects of PEG-rHuMGDF on pharmacokinetics, megakaryocytopoiesis, platelet production, and platelet function were characterized for dosing 0.05, 0.10, 0.50, or 2.5 mu g/kg/d in 22 baboons for 28 days. Daily subcutaneous injections of PEG-rHuMGDF produced linear log-dose responses in (1) steady-state trough plasma levels of PEG-HuMGDF (P < 10(-3)); (2) marrow megakaryocyte volume (P < 10(-3)), ploidy (P < 10(-4)), and number (P < .01); and (3) peripheral platelet concentrations (P < 10(-4)) and platelet mass turnover (P < 10(-3)). Platelet morphology, life span, and recovery were normal, and peripheral leukocyte, neutrophil, and erythrocyte counts were not significantly affected by PEG-rHuMGDF (P > .1 in all cases). PEG-rHUMGDF at 0.5 mu g/kg/d produced similar blood concentrations of %Mpl%-ligand and platelets as 10 times the dose of rHuMGDF (5.0 mu g/kg/d), reflecting the extended plasma half-life achieved through pegylation. Whereas PEG-rHuMGDF did not induce platelet aggregation in vitro, platelet aggregatory responsiveness induced by thrombin receptor %agonist% peptide (TRAP(1-6)) and collagen was transiently enhanced ex vivo during the initial few days of PEG-rHuMGDF administration. However, adenosine diphosphate (ADP)-induced platelet aggregation was not enhanced ex vivo by PEG-rHuMGDF therapy. In-111-platelet deposition on segments of homologous endarterectomized aorta (EA) and vascular graft (VG) interposed in arteriovenous femoral shunts increased in direct proportion to the circulating platelet concentration (P < 10(-4) for both EA and VG); I-125-fibrin accumulation was not affected by PEG-rHuMGDF-induced increases in peripheral platelet counts. Changes in platelet production and function produced by PEG-rHuMGDF returned to baseline within 2 weeks after discontinuing treatment. Thus, in nonhuman primates, PEG-rHuMGDF increases platelet production in a linear log-dose-dependent manner by stimulating megakaryocyte endoreduplication and new megakaryocyte formation from marrow hematopoietic progenitors. These findings suggest that appropriate dosing of PEG-rHuMGDF therapy during periods of chemotherapy-induced marrow suppression may maintain hemostatic concentrations of peripheral platelets without increasing the risk of thrombosis. (C) 1996 by The American Society of Hematology.

2/3,AB/14 (Item 9 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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04625964 Genuine Article#: TY339 Number of References: 67 Title: REGULATION OF PLATELET PRODUCTION AND FUNCTION BY MEGAKARYOCYTE GROWTH AND DEVELOPMENT FACTOR IN NONHUMAN-PRIMATES
Author(s): HARKER LA; HUNT P; MARZEC UM; KELLY AB; TOMER A; HANSON SR; STEAD RB
Corporate Source: EMORY UNIV,SCH MED,DIV HEMATOL & ONCOL,YERKES REG PRIMATE RES CTR,PO DRAWER AR/ATLANTA//GA/30322; AMGEN INC/THOUSAND OAKS//CA/91320
Journal: BLOOD, 1996, V87, N5 (MAR 1), P1833-1844
ISSN: 0006-4971
Language: ENGLISH Document Type: ARTICLE
Abstract: The primary physiologic regulator of platelet production, %Mpl% ligand, has recently been cloned and characterized. To define the regulatory role of %Mpl% ligand on platelet production and function we measured the effects of a recombinant truncated human %Mpl% ligand, megakaryocyte growth and development factor (rHu-MGDF) on megakaryocytopoiesis. platelet function, and thrombogenesis in nonhuman primates. rHu-MGDF was administered to 10 baboons for 28 days while performing pharmacokinetics and repeated measurements of the following: (1) platelet count, volume, turnover, and function ex vivo and in vitro; (2) marrow megakaryocyte number, volume, and ploidy; and (3) platelet deposition and fibrin accumulation on segments of vascular graft and endarterectomized aorta in vivo. Daily subcutaneous injections of rHU-MGDF (5 mu g/kg/d) attained plasma concentrations averaging 1,300 +/- 300 pg/mL 2 hours after injection with trough levels of 300 +/- 65 pg/mL before the next dose. These levels of rHu-MGDF incrementally increased the peripheral platelet concentration threefold by day 7 and fivefold by day 28 (P < 10(-4)) associated with a reciprocal decrease of 25% in mean platelet volumes (P < 10(-3)). Platelet mass turnover, a steady-state measure of platelet production, increased fivefold (P < 10(-4)). Platelet morphology, life span, and recovery were normal. No significant change occurred in peripheral leukocyte, neutrophil, or erythrocyte counts (P > .1 in all cases). The platelet count gradually returned to baseline within 2 weeks after discontinuing rHu-MGDF injections. Marrow megakaryocyte volume doubled (P < 10(-3)) three days after initiating rHU-MGDF therapy and the modal ploidy shifted from 16N to 64N (P < 10(-4)). Marrow megakaryoctye number increased twofold by day 7, and nearly fourfold by day 28 (P < 10(-4)), resulting in a 6.5-fold increase in marrow megakaryocyte mass (P < 10(-3)). The effects of rHu-MGDF on thrombosis were determined by comparing baseline, day 5, and day 28 rHU-MGDF-treatment measurements of (111)n-platelet deposition and I-125-fibrin accumulation on segments of homologous endarterectomized aorta (EA) and vascular graft (VG) interposed in arteriovenous femoral shunts. rHu-MGDF increased In-111-platelet

deposition in direct proportion to the circulating concentration of platelets for both EA and VG (r = .98 in both cases), without significant changes in fibrin accumulation (P > .5 in both cases). During the first week of rHu-MGDF treatment ex vivo platelet aggregatory responsiveness was enhanced to physiologic %agonists% (adenosine diphosphate, collagen, and thrombin receptor %agonist% peptide, TRAP(1-6)) (P < .05 in all cases). Although in vitro platelet aggregation was not induced by any concentration of rHu-MGDF tested (P > .5), rHu-MGDF enhanced aggregatory responses to low doses of physiologic %agonists%, effects that were maximal at 10 ng/mL for baboon platelets and 100 ng/mL for human platelets, and were blocked by excess soluble c-%Mpl% receptor. Flow cytometric expression of platelet activation epitopes was not increased on resting platelets (ligand-induced binding sites, P-selectin, or Annexin V binding sites; P > .1 in all cases). Megakaryocyte growth and development factor regulates platelet production and function by stimulating endoreduplication and megakaryocyte formation from marrow progenitor cells, and transiently enhancing platelet functional responses ex vivo. rHu-MGDF has the potential for achieving platelet hemostatic protection with minimal thrombo-occlusive risk. (C) 1996 by The American Society of Hematology.
? s mpl

S3 2151 MPL
? s antibody or antibodies

504850 ANTIBODY
537321 ANTIBODIES
S4 811757 ANTIBODY OR ANTIBODIES
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Set Items Description
S1 19 MPL AND (ANTIBODY OR ANTIBODIES) AND AGONIST? S2 14 RD (unique items)
S3 2151 MPL
S4 811757 ANTIBODY OR ANTIBODIES
? s s3 and s4 not s1

2151 S3
811757 S4
19 S1
S5 314 S3 AND S4 NOT S1
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Processing
314 S5
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S6 220 S5 AND PY<1998
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...examined 50 records (150)
...examined 50 records (200)
...completed examining records
S7 153 RD (unique items)
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7/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09897921 99182300
A prospective trial of steroid cessation after renal transplantation in pediatric patients treated with cyclosporine and mizoribine. Motoyama O; Hasegawa A; Ohara T; Hattori M; Kawaguchi H; Takahashi K; Kamiyama Y; Nakai H; Shishido S; Ogawa O; Kawamura T; Tsuzuki K; Oshima S First Department of Pediatrics, Toho University School of Medicine, Tokyo, Japan.
Pediatr Transplant (DENMARK) Aug %1997%, 1 (1) p29-36, ISSN 1397-3142 Journal Code: C8X
Languages: ENGLISH
Document type: CLINICAL TRIAL; JOURNAL ARTICLE; MULTICENTER STUDY We conducted a multi-center prospective study to evaluate the safety and efficacy of steroid withdrawal after renal transplantation in children. In 52 children (51 living-related donor transplants and 1 cadaver donor transplant), immunosuppressive therapy was started with cyclosporine (CyA), mizoribine (MZ), methylprednisolone (%MPL%) and anti-lymphocyte globulin. Administration of %MPL% was reduced to alternate days more than 6 months after transplantation, and attempts were made to withdraw it. Acute rejection was noted in 19 patients (36.5%) by 1 month after transplantation. The whole-blood CyA trough level using monoclonal %antibody% was 175.0+/-17.0 ng/ml in patients who developed acute rejection and 282.0+/-25.3 ng/ml in those who did not show acute rejection (p<0.01). During the 37 attempts at alternate-day %MPL% administration, clinical acute rejection was observed in only 1 patient and chronic rejection in 3. During 10 attempts to withdraw %MPL% , acute rejection was noted in 3 patients, but graft function recovered to the pre-rejection level after treatment of the acute rejection. At the last observation, graft function was lost in 3 patients, 22 were receiving %MPL% on alternate days, and %MPL% had been withdrawn from 7 for a mean period of 16.7 months. The survival rate of the patients and the grafts was 100% and 94% after an average follow-up period of 4 years. Evaluation of growth showed catch-up growth in all patients during the withdrawal period.

7/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09471259 98163421
Mesenteric and portal venous obstruction associated with primary antiphospholipid %antibody% syndrome [see comments]
Lee HJ; Park JW; Chang JC
Department of Internal Medicine, Yeungnam University College of Medicine, Namgu, Taegu, Korea.
J Gastroenterol Hepatol (AUSTRALIA) Dec %1997%, 12 (12) p822-6, ISSN 0815-9319 Journal Code: A6J
Comment in J Gastroenterol Hepatol 1997 Dec;12(12):827-8 Languages: ENGLISH
Document type: JOURNAL ARTICLE
A 58-year-old Korean man who had a past history of appendicitis, superior mesenteric vein thrombosis and intestinal obstruction presented 7 years later suffering from colicky right upper quadrant pain, epigastric discomfort after fatty meals, nausea and vomiting. He was found to have thrombosis of the superior mesenteric and portal veins, portal hypertension with oesophageal varices, cholangitis, and a biliary stone. The serum anti-cardiolipin %antibody% (aCL) titres were 103 immunoglobulin (Ig)G antiphospholipid units (GPL) and 50 IgM antiphospholipid units (%MPL%) and the aCL-IgG titre was still high at 106 2 years after the initial diagnosis. No evidence of disease states known to be associated with antiphospholipid %antibodies% was found. We report a patient with mesenteric and portal venous obstruction associated with the primary antiphospholipid syndrome (APS).

7/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09457085 98138784
Anticardiolipin %antibodies% in pre-eclampsia and intrauterine growth retardation.
D'Anna R; Scilipoti A; Leonardi J; Scuderi M; Jasonni VM; Leonardi R Institute of Gynecology, University of Messina, Italy.
Clin Exp Obstet Gynecol (ITALY) %1997%, 24 (3) p135-7, ISSN 0390-6663 Journal Code: DB1
Languages: ENGLISH
Document type: JOURNAL ARTICLE
OBJECTIVE: To establish an association of anticardiolipin %antibody% (ACA) levels and pre-eclampsia or intrauterine growth retardation (IUGR). METHODS: Twenty-eight patients with pre-eclampsia, 28 with IUGR and 28 normotensive control group were matched for maternal age, race, weight, cigarette smoking, and parity. All had plasma anticardiolipin %antibodies% (GPL and %MPL%) detected by the modified enzyme-linked immuno-absorbent assay (ELISA) technique. RESULTS: No statistical significant difference in ACA values, both GPL and %MPL%, was found among the three groups studied Furthermore, none reached a value of ACA that could be considered clinically relevant (> 15). CONCLUSION: No association was found in anticardiolipin %antibody% levels between pre-eclamptic and IUGR versus the control group.

7/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09434323 98115543
Value of anticardiolipin %antibodies% for monitoring disease activity in systemic lupus erythematosus and other rheumatic diseases. Buttgereit F; Grunewald T; Schuler-Maue W; Burmester GR; Hiepe F Department of Medicine III, Rheumatology and Clinical Immunology, Charite University Hospital, Humboldt-Universitat, Berlin, Federal Republic of Germany.
Clin Rheumatol (BELGIUM) Nov %1997%, 16 (6) p562-9, ISSN 0770-3198 Journal Code: DI6
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The prevalence of anticardiolipin %antibodies% in active systemic lupus erythematosus (SLE) was compared with that in inactive SLE and other rheumatic and non-rheumatic diseases to determine the value of these autoantibodies in monitoring rheumatic diseases. Pairs of IgG- and IgM-aCL were measured by ELISA in 173 consecutive hospitalised patients, including 141 with rheumatic diseases (18 active SLE, 21 inactive SLE, 19 rheumatoid arthritis, 13 reactive arthritis, 7 other spondyloarthropathies, 16 vasculitis, 47 other autoimmune diseases) and 32 non-rheumatic controls. A further 101 aCL pairs were determined during follow-up in 19 patients with SLE. Serum concentrations were analysed with respect to SLE activity and compared between the different patient groups. IgG- and IgM-aCL levels in excess of 10 GPL and 9 %MPL% respectively were considered positive. 30.6% of all patients (53/173) were found to be positive for IgG-aCL, as against only 9.8% (17/173) for IgM-aCL. IgG-aCL serum levels in active SLE differed significantly from all other groups, including inactive SLE (all $p < 0.005$). Median IgM-aCL levels were below the cut off point in all groups, although measurable values were obtained almost exclusively in active SLE and RA. In this study IgM-aCL measurement was of less value in monitoring rheumatic diseases. IgG-aCL positivity in SLE was associated with a significantly higher odds ratio (OR) for active disease (OR 16.0, 95% confidence interval: 2.8-90.0). The results show that disease activity

in SLE was accompanied by significantly increased IgG-aCL, whereas no elevation was found in other diseases. This parameter may therefore be useful in monitoring SLE activity.

7/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09392056 98031261
The muramyl dipeptide analog GMTP-N-DPG preferentially induces cellular immunity to soluble antigens.
Fast DJ; Vosika GJ
Endorex Corporation, Fargo, ND 58104, USA.
Vaccine (ENGLAND) Nov %1997%, 15 (16) p1748-52, ISSN 0264-410X Journal Code: X60
Languages: ENGLISH
Document type: JOURNAL ARTICLE
GMTP-N-DPG (N-acetylglucosaminyl-N-acetylmuramyl-L-alanyl-D-isoglutamyl- L-alanyl-dipalmitoylpropylamide) is a lipophilic derivative of the immunologically active compound MDP and has adjuvant properties. GMTP-N-DPG was compared with other adjuvants in model vaccine systems using ovalbumin (OVA) and a synthetic peptide derived from pp89 of murine cytomegalovirus as antigens. When serum from C57/Bl mice immunized with OVA was tested for the presence of anti-OVA %antibody%, samples from mice immunized with OVA plus GMTP-N-DPG had ELISA optical density (O.D.) readings twice as high as those from mice immunized with antigen alone. In contrast, samples from mice immunized with the liposomal monophosphoryl lipid A (%MPL%) formulation exhibited ELISA O.D. readings tenfold higher than samples from mice immunized with antigen alone. Relative levels of specific %antibody% in serum samples from mice immunized with OVA plus the saponin adjuvant QS-21 were equal to the GMTP-N-DPG samples. When spleen cells from immunized mice were tested for their proliferative response to OVA, we found that liposomal %MPL% was again the optimal adjuvant, whereas the proliferative responses of cells from mice immunized with GMTP-N-DPG or QS-21 were no better than cells from mice immunized with OVA alone. In contrast to the relatively low %antibody% and proliferation levels, spleen cells from mice immunized with GMTP-N-DPG and OVA demonstrated the highest level of anti-OVA CTL activity. Spleen cells from mice immunized with the pp89 peptide plus GMTP-N-DPG also exhibited CTL activity. Using %antibody% and complement mediated cytotoxicity it was determined that the CTL were CD8+. Based on these results, we believe that GMTP-N-DPG may be an excellent candidate adjuvant in vaccines for diseases in which a strong cell-mediated response is desired.

7/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09376099 98094951
Thrombopoietin and erythropoietin activate inside-out signaling of integrin and enhance adhesion to immobilized fibronectin in human growth-factor-dependent hematopoietic cells.
Gotoh A; Ritchie A; Takahira H; Broxmeyer HE
Department of Microbiology and Immunology, Walther Oncology Center, Indiana University School of Medicine, Indianapolis 46202, USA. Ann Hematol (GERMANY) Nov-Dec %1997%, 75 (5-6) p207-13, ISSN 0939-5555 Journal Code: A2P
Contract/Grant No.: R01 HL54037, HL, NHLBI; R01 HL46416, HL, NHLBI; P01 HL53586, HL, NHLBI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Erythropoietin (EPO) and thrombopoietin (c-%MPL% ligand; TPO) are structurally similar cytokines and support respectively, the proliferation and differentiation for erythroid and megakaryocytic lineages, as well as more primitive progenitors. We studied the effect of these cytokines on the induction of adhesion of human growth-factor-dependent hematopoietic cells to immobilized fibronectin, which is a main component of the extracellular matrix in the bone marrow. MO7ER cells that are genetically engineered to express human EPO receptor and MO7e cells that express endogenous c-%MPL% were used. Stimulation with either TPO or EPO induced rapid increases in adhesion of MO7ER cells to fibronectin without apparent change of expression of integrins. Experiments with inhibitory monoclonal %antibodies% (mAbs) demonstrated that CD41, which has been reported to be involved in TPO-induced adhesion of megakaryocytic cells, is not responsible for this enhanced adhesion. Anti-beta 1 integrin mAb inhibited adhesion completely, while inhibition by anti-alpha 4 integrin mAb and anti-alpha 5 integrin mAb was partial. Combination of anti-alpha 4 mAb plus anti-alpha 5 mAb completely abolished adhesion, as did anti-beta 1 mAb, suggesting that the adhesion is mediated by both alpha 4 beta 1 and alpha 5 beta 1 integrins. Experiments using inhibitors suggested that ligand binding followed by activation of intracellular tyrosine kinases along with PI3-kinase activation is required. After stimulation of MO7ER cells with either TPO or EPO, fibronectin-attached cells, but not cells in suspension, showed tyrosine phosphorylation of focal adhesion kinase, which plays a central role in integrin-mediated signaling. These data suggest that TPO and EPO might

be involved in homing/migration to the bone marrow microenvironment by hematopoietic cells that express corresponding receptors.

7/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09349571 98054108
Synthetic lipopeptides incorporated in liposomes: in vitro stimulation of the proliferation of murine splenocytes and in vivo induction of an immune response against a peptide antigen.
Fernandes I; Frisch B; Muller S; Schuber F
Laboratoire de Chimie Bioorganique (URA CNRS 1386), Universite Louis Pasteur, Illkirch, France.
Mol Immunol (ENGLAND) Jun %1997%, 34 (8-9) p569-76, ISSN 0161-5890 Journal Code: NG1
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Amphiphilic lipopeptides, such as Pam3CysAlaGly and Pam3CysSerSer, were synthesized and incorporated into liposomes, and their ability to induce the proliferation of BALB/c mouse splenocyte was tested in vitro. When compared to monophosphoryl lipid A (%MPL%) the following potency order was found: liposomal lipopeptides > liposomal %MPL% > free (emulsified) lipopeptides. These results strongly depend on the size of the vesicles used: a mitogenic effect was observed only with lipopeptides incorporated within vesicles of diameter < or = 100 nm while lipopeptides in larger vesicles (diameter approximately 300 nm) gave no response. This may be related to the necessity for the liposome-associated lipopeptides to be endocytosed to reach putative intracellular targets. As immunoadjuvanticity seems to be linked to B-lymphocyte activation, the lipopeptides represent attractive alternatives to %MPL% for the realization of completely synthetic liposome-based peptide vaccine formulations. This was borne out by showing that Pam3CysAlaGly and Pam3CysSerSer, when incorporated in small unilamellar vesicles carrying a covalently conjugated synthetic peptide of sequence IRGERA, corresponding to an epitope of the C-terminal region of histone H3, were able to induce a potent and long-lasting immune response.

7/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09339753 98052531
Simultaneous activation of signals through gp130, c-kit, and interleukin-3 receptor promotes a trilineage blood cell production in the absence of terminally acting lineage-specific factors.
Kimura T; Sakabe H; Tanimukai S; Abe T; Urata Y; Yasukawa K; Okano A; Taga T; Sugiyama H; Kishimoto T; Sonoda Y
Department of Hygiene, Kyoto Prefectural University of Medicine, Kyoto, Japan.
Blood (UNITED STATES) Dec 15 %1997%, 90 (12) p4767-78, ISSN 0006-4971 Journal Code: ABG
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We assessed the biologic role of signaling through gp130, a signal-transducing receptor (R) component, in human hematopoiesis in vitro. Although peripheral blood-derived CD34(+) cells ubiquitously expressed gp130 and interleukin-3 receptor alpha (IL-3Ralpha), IL-6Ralpha was only detected on 80% of these CD34(+) cells. We sorted CD34(+)IL-6R+ or CD34(+)IL-6R- cells and studied the effect on hematopoietic colony formation of signaling through gp130 activated by IL-6 or a combination of IL-6 and recombinant soluble human IL-6R (sIL-6R) in the presence or absence of stem cell factor (SCF) and/or IL-3. Signals activated by SCF, IL-6, or IL-6/sIL-6R complex alone did not induce significant colony formation. However, a combination of IL-3, SCF, and IL-6/sIL-6R complex dramatically induced many neutrophil (colony-forming unit-granulocyte [CFU-G]), erythroid burst (burst-forming unit-erythrocyte [BFU-E]), erythrocyte-containing mixed (CFU-Mix), and megakaryocyte (CFU-Meg) colony formations when CD34(+)IL-6R- cells were used as the target. CFU-G colony formation induced by the three signals was more evident when CD34(+)IL-6R+ cells were used as the target. This distinct synergistic effect of the three different signals was confirmed by single-cell clone-sorting experiments. Moreover, colony formation (including CFU-G, BFU-E, CFU-Mix, and CFU-Meg) was observed even in the presence of neutralizing %antibodies% for granulocyte colony-stimulating factor, erythropoietin, and thrombopoietin (c-%Mpl%), whereas neutralizing %antibodies% for gp130, IL-6R, IL-3, and SCF partially or completely blocked the synergistic effect. The maturation of neutrophilic, erythroid, and megakaryocytic cells supported by the three signals in serum-free cultures was confirmed by immunostaining using anti-CD66b, antiglycophorin A, antihemoglobin alpha, and anti-CD41 monoclonal %antibodies%, respectively. In contrast, any two of the three signals were insufficient for effective blood cell production in the absence of maturation factors. These results suggest that simultaneous activation of the three signals through gp130, c-kit, and IL-3R can induce in vitro proliferation and differentiation of trilineage hematopoietic progenitors in the absence of terminally acting lineage-specific factors.

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7/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09315099 98031001

Serological characteristics of systemic lupus erythematosus from a hospital-based rheumatology clinic in Kuwait.

al-Mekaimi A; Malaviya AN; Serebour F; Umamaheswaran I; Kumar R; al-Saeid K; Sharma PN

Department of Microbiology (Clinical Immunology Division), Faculty of Medicine, Kuwait University, Kuwait.

Lupus (ENGLAND) %1997%, 6 (8) p668-74, ISSN 0961-2033 Journal Code: BRN

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Thirty-one consecutive patients with SLE were screened for antinuclear %antibody% (ANA), anti-DNA %antibodies%, extractable nuclear antigen %antibodies% (anti-ENAs) including anti-Sm, anti-RNP, anti-SSA (anti-Ro), anti-SSB; (anti-La), anti-Scl-70, rheumatoid factor (RF), C-reactive protein (CRP), C3 and C4 levels, anti-cardiolipin %antibodies% (aCL), biologically false positive serological test for syphilis (BF-STS) using VDRL test and Coombs' test. The age of the patients ranged from 11 to 52 year with a median of 29 year; female to male ratio of 5:1. There were 21 Kuwaitis, four Egyptians, three from the Indian subcontinent, two Filipinos and one Syrian. Main clinical categories of SLE were: mild cutaneous SLE in 12 (38.7%), clinical antiphospholipid syndrome (APS) secondary to SLE in 8 (25.8%), haematological manifestations of SLE in 5 (16.1%), renal lupus in four (12.9%), neuropsychiatric in three (9.7%), others (6.4%). Clinical features overlapped in several patients. ANA was positive in 96.8% (mean value 891.61 units/ml), anti-DNA in 35.5% (mean value 56.4 units) that was lower than expected and could be due to selection bias as the patients were from a rheumatology clinic, anti-ENA in 42%, anti-Sm 13% that was lower than other non-Caucasian populations, anti-RNP 13%, anti-SS-A in 35.5%, anti-SS-B in 19.4%, Scl-70 in 13%, CRP in 71% (moderate 58%, very high 13%); C3 mean 1.52 mg/ml (3.2% low levels), C4 mean 0.35 mg/ml (32% low levels), anticardiolipin mean GPL 35.35 units (high 58%), mean %MPL% 10.61 units (high 26%), BF-STS in 6%, Coombs' test in 6%, RF positive in 36%. The only significant positive clinical associations observed were those of renal involvement with anti-DNA %antibodies% (P = 0.042), and clinical antiphospholipid %antibody% syndrome with aCL %antibodies% (P = < 0.05).

7/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09313753 98022797

Markedly reduced expression of platelet c-%mpl% receptor in essential thrombocythemia.

Horikawa Y; Matsumura I; Hashimoto K; Shiraga M; Kosugi S; Tadokoro S; Kato T; Miyazaki H; Tomiyama Y; Kurata Y; Matsuzawa Y; Kanakura Y Department of Internal Medicine II, Osaka University Medical School, Osaka, Japan.

Blood (UNITED STATES) Nov 15 %1997%, 90 (10) p4031-8, ISSN 0006-4971 Journal Code: ABG

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Thrombopoietin (TPO) is implicated as a primary regulator of megakaryopoiesis and thrombopoiesis through binding to the cytokine receptor c-%Mpl% (the product of the c-%mpl% proto-oncogene). In an effort to determine the pathophysiological role of TPO-c-%Mpl% system in essential thrombocythemia (ET), we have examined the levels of serum TPO and the expression and function of platelet c-%Mpl% in 17 patients with ET. In spite of extreme thrombocytosis, serum TPO levels were slightly elevated or within normal range in most, if not all, patients with ET (mean +/- SD, 1.31 +/- 1.64 fmol/mL), as compared with normal subjects (0.76 +/- 0.21 fmol/mL). Flow cytometric and Western blot analyses revealed that the expression of platelet c-%Mpl% was strikingly reduced in all patients with ET. Furthermore, the expression of platelet c-%mpl% mRNA was found to be significantly decreased in the ET patients tested. In contrast, almost identical levels of GPIIb/IIIa protein and mRNA were expressed in platelets from ET patients and normal controls. In addition to expression level, activation state of platelet c-%Mpl% was investigated in ET patients. Immunoblotting with anti-phosphotyrosine %antibody% showed that no aberrant protein-tyrosine phosphorylation was observed in platelets of ET patients before treatment with TPO, and the levels of TPO-induced protein-tyrosine phosphorylation, including c-%Mpl% -tyrosyl phosphorylation, roughly paralleled those of c-%Mpl% expression, suggesting that c-%Mpl%-mediated signaling pathway was not constitutively activated in platelets of ET patients. These results suggested that the TPO-c-%Mpl% system may not be directly linked to pathogenesis of ET, and that gene(s) mutated in ET may be important in regulating the levels of c-%mpl% gene expression in addition to the growth and differentiation of multipotential hematopoietic stem cells.

7/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09282954 98008110

Chronic thrombocytopenia is induced in dogs by development of cross-reacting %antibodies% to the %MpL% ligand.
Dale DC; Nichol JL; Rich DA; Best DM; Slichter SJ; Sheridan W; Hunt P Department of Medicine, University of Washington and the Puget Sound Blood Center, Seattle, WA 98195-6422, USA.
Blood (UNITED STATES) Nov 1 %1997%, 90 (9) p3456-61, ISSN 0006-4971 Journal Code: A8G
Contract/Grant No.: RO1-18951
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The %MpL% ligand (ML) is a potent stimulus for thrombocytopoiesis. To create an in vivo model of ML deficiency, we injected dogs with a recombinant human ML (rhML) to determine whether cross-reacting %antibodies% would develop and cause thrombocytopenia. RhML was administered subcutaneously for 8 weeks to three normal dogs (mean platelets, 197 +/- 5.5 x 10(3)/microL). Within 5 days their platelet counts were twice baseline and greater than 4 times baseline by day 21. Then, uniformly, chronic thrombocytopenia developed. At 1 week after terminating rhML, mean platelets were 0.5 times baseline and at 2 months 0.25 times baseline. Early in treatment, marrow biopsies showed increased megakaryocyte number and ploidy, which decreased as platelets declined. Paralleling these changes, high titer anti-rhML %antibodies% developed. Autologous 51Cr-labeled platelet recovery and survival measurements indicated that the thrombocytopenia was principally due to decreased production. Infusion of plasma from the thrombocytopenic dogs into two normal dogs and one dog previously made thrombocytopenic with rhML caused platelet counts to fall gradually. These studies show that dogs with anti-rhML %antibodies% develop thrombocytopenia, presumably because the cross-reacting %antibodies% neutralize endogenous canine ML. The results strongly suggest that ML plays an essential role in maintaining normal platelet levels.

7/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09198196 97400151
Anticardiolipin %antibodies% are not associated with restenosis or endothelial activation after percutaneous transluminal angioplasty. Tsakiris DA; Tschopl M; Jager K; Wolf F; Marbet GA
Department of Central Laboratory, University Hospital Basel, Switzerland. Int Angiol (ITALY) Jun %1997%, 16 (2) p88-93, ISSN 0392-9590 Journal Code: INE
Languages: ENGLISH
Document type: JOURNAL ARTICLE
OBJECTIVE: Restenosis following percutaneous transluminal angioplasty (PTA) continues to be a major clinical problem. Anticardiolipin %antibodies% (aCL) have been established as risk factors for venous or arterial thrombosis. The aim of this study was to assess: a) the influence of positive aCL upon restenosis within 6 months after PTA, b) the possibility of a seroconversion from negative to positive aCL after PTA and c) a possible link between positive aCL and endothelial activation. EXPERIMENTAL DESIGN: 71 patients (50 men and 21 women, age 68+/-13 years) with peripheral arterial occlusive disease (PAOD, Fontaine II-IV) undergoing a successful PTA entered the study and were prospectively followed for 3 and 6 months thereafter. INTERVENTIONS: PTA was carried out successfully and noninvasive grading was done with duplex scanning. Laboratory investigation included aCL, thrombin generation markers, such as thrombin-antithrombin III complexes and prothrombin fragments 1+2, as well as thrombomodulin, soluble P-selectin, E-selectin and the vascular cell adhesion molecule-1, as endothelial activation markers. RESULTS: 30/71 (42.3%) patients developed restenosis (>50% reduction of the lumen diameter) within 6 months after PTA. 9/71(12.7%), had positive aCL IgG (19-35 GPL) and/or IgM (14-103 %MPL%) at all three measurements. 2/9 (22.2%) of aCL positive and 28/62 (45.2%) of aCL negative patients had restenosis at 6 months after PTA (relative risk RR=0.51, 95%-CI: 0.14-1.78, chi2 non-significant). All other parameters did not differ between aCL positive and -negative groups. CONCLUSIONS: Our findings suggest that: a) patients with PAOD have a slightly higher prevalence of positive aCL compared to the general population, but no association is evident between positive aCL and restenosis within 6 months after PTA, b) no seroconversion from negative to positive aCL occurred within 6 months after PTA, c) no association of aCL with endothelial activation markers or thrombin generation markers was found.

7/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09196787 97438124
Recombinant human c-%Mpl% ligand (thrombopoietin) not only acts on megakaryocyte progenitors, but also on erythroid and multipotential progenitors in vitro.
Tanimukai S; Kimura T; Sakabe H; Ohmizono Y; Kato T; Miyazaki H; Yamagishi H; Sonoda Y
Second Department of Surgery, Kyoto Prefectural University of Medicine, Kamigyoku, Japan.
Exp Hematol (UNITED STATES) Sep %1997%, 25

(10) p1025-33, ISSN 0301-472X Journal Code: EPR
Languages: ENGLISH
Document type: JOURNAL ARTICLE
To determine the hematopoietic actions of recombinant human c-%Mpl% ligand (thrombopoietin [TPO]), we studied its effects on the proliferation and differentiation of highly purified CD34+ blood progenitors in plasma-containing and serum-free culture. TPO alone promoted the growth of small megakaryocyte colonies (CFU-Meg) in numbers two to three times greater than those produced by interleukin (IL)-3. The combination of TPO and stem cell factor (SCF) exerted a significant synergistic effect on CFU-Meg formation. In the presence of TPO and IL-3 or granulocyte/macrophage-colony stimulating factor (GM-CSF), a significant number of mixed colonies (CFU-Mix) were observed. The combination of TPO and Epo did not increase the number of CFU-Meg, but did support erythroid-burst (BFU-E) and CFU-Mix colony formation. Interestingly, the combination of TPO with cytokines known to have burst-promoting activity (BPA), including IL-3, GM-CSF, IL-9, and SCF, increased the number of BFU-E and CFU-Mix in the presence of Epo. The BPA of TPO was further investigated by delayed addition of Epo on day 6 after incubation with TPO from day 0. None of the BFU-E or CFU-Mix survived, indicating that TPO acted as a costimulant exclusively for Epo. Moreover, a neutralizing anti-human %Mpl% receptor polyclonal %antibody% completely abrogated the BPA of TPO, demonstrating that this effect was mediated through the %Mpl% receptor. Finally, experiments in single-cell clone sorting and serum-free culture clearly demonstrated that a combination of TPO and Epo directly supported BFU-E and CFU-Mix. These results suggest that TPO acts not only in megakaryocytopoiesis but also in the early stage of hematopoiesis.

7/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09188751 97427927
Monophosphoryl lipid A enhances both humoral and cell-mediated immune responses to DNA vaccination against human immunodeficiency virus type 1. Sasaki S; Tsuji T; Hamajima K; Fukushima J; Ishii N; Kaneko T; Xin KQ; Mohri H; Aoki I; Okubo T; Nishioka K; Okuda K
Department of First Internal Medicine, Yokohama City University School of Medicine, Yokohama, Japan.
Infect Immun (UNITED STATES) Sep %1997%, 65 (9) p3520-8, ISSN 0019-9567 Journal Code: GO7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
To enhance immunity induced by DNA vaccination against human immunodeficiency virus type 1 (HIV-1), we evaluated the efficacy of monophosphoryl lipid A (%MPL%), an adjuvant of bacterial origin. BALB/c mice were intramuscularly injected with immunogenic DNA, encoding the env and rev genes of the HIV-1(IIIB) strain, formulated with %MPL% dissolved in different vehicles (%MPL% in stable emulsion and %MPL% in aqueous formulation). The sera from mice immunized with the two preparations of %MPL% revealed 2(6) to 2(9) times higher HIV-1-specific immunoglobulin G (IgG) titers than the sera from mice immunized without %MPL%. In virus neutralization tests for HIV-1(IIIB), by p24 assay and antifusion assay of infected MOLT-4 cells, %MPL% tends to elicit %antibody% more protective than %antibody% elicited without adjuvant. %MPL% also elicited stronger delayed-type hypersensitivity and cytotoxic-T-lymphocyte activity against HIV-1(IIIB) compared to DNA alone. HIV-1-specific IgG subclass analysis showed that %MPL% tends to facilitate IgG2a production, suggesting enhancement of a predominant T-helper-type-1 response, and this enhancement may help to facilitate protective-%antibody% induction. Furthermore, a chloramphenicol acetyltransferase (CAT) assay was employed to determine whether %MPL% affected the gene expression process. Interestingly, both %MPL% preparations reduced CAT activity in the muscle injected with CAT expression vector but increased anti-CAT %antibody% production. These results indicate that %MPL% acts as an effective adjuvant for immunogenic DNA injection despite reduced expression of encoding protein in muscle. We conclude that %MPL% has a strong adjuvant effect on DNA vaccination against HIV-1.

7/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09177405 97410202
Thrombopoietin alone stimulates the early proliferation and survival of human erythroid, myeloid and multipotential progenitors in serum-free culture.
Yoshida M; Tsuji K; Ebihara Y; Muraoka K; Tanaka R; Miyazaki H; Nakahata T
Department of Clinical Oncology, Institute of Medical Science, University of Tokyo, Japan.
Br J Haematol (ENGLAND) Aug %1997%, 98 (2) p254-64, ISSN 0007-1048 Journal Code: AXC
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We examined the effects of recombinant human thrombopoietin (TPO, c-%Mpl% ligand) on the proliferation and differentiation of human haemopoietic progenitors other than megakaryocytic progenitors using serum-free cultures. TPO alone supported the generation of not only megakaryocytic (MK) but also

blast cell (blast) colonies from cord blood CD34+ cells. Delayed addition of a cytokine cocktail (cytokines; interleukin (IL)-3, IL-6, stem cell factor, erythropoietin, granulocyte-macrophage colony-stimulating factor, and TPO) to cultures with TPO alone on day 7 induced various colonies including granulocyte-macrophage (GM) colonies, erythroid bursts (E), granulocyte-erythrocyte-macrophage-megakaryocyte (GEMM) colonies. Replating experiments of blast colonies supported by TPO alone for culture with cytokines revealed that approximately 60% of the blast colonies contained various haemopoietic progenitors. Single cell cultures of clone-sorted CD34+ cells indicated that TPO supported the early proliferation and/or survival of both primitive and committed haemopoietic progenitors. In serum-free suspension cultures, TPO alone significantly stimulated the production of progenitors for MK, GM, E and GEMM colonies as well as long-term culture-initiating cells. These effects were completely abrogated by anti-TPO %antibody% . These results suggest that TPO is an important cytokine in the early proliferation of human primitive as well as committed haemopoietic progenitors, and in the ex vivo manipulation of human haemopoietic progenitors.

7/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09165364 97400539
Mutational analysis of thrombopoietin for identification of receptor and neutralizing %antibody% sites.
Pearce KH Jr; Potts BJ; Presta LG; Bald LN; Fendly BM; Wells JA Department of Protein Engineering, Genentech, Inc., South San Francisco, California 94080, USA.
J Biol Chem (UNITED STATES) Aug 15 %1997%, 272 (33) p20595-602, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Thrombopoietin (TPO) is a hematopoietin important for megakaryocyte proliferation and production of blood platelets. We sought to characterize how TPO binds and activates its receptor, myeloproliferative leukemia virus receptor. The erythropoietin-like domain of TPO (TPO1-153) has been fused to the gIII coat protein of M13 bacteriophage. Forty residues were chosen for mutation to alanine using the criteria that they were charged residues or predicted to be solvent-exposed, based on a homology model. Phage enzyme-linked immunosorbent assay was used to determine affinities for binding to both the TPO receptor and five anti-TPO1-153 monoclonal %antibodies%. Mutations at mostly positively charged residues (Asp8, Lys14, Lys52, Lys59, Lys136, Lys138, Arg140) caused the greatest reduction in receptor-binding affinity. Most of these residues mapped to helices-1 and -4 and a loop region between helix-1 and helix-2. Two of the monoclonal %antibodies% that blocked TPO binding and bioactivity had determinants in helix-4. In contrast, the other three monoclonal %antibodies%, which were effective at blocking TPO activity but did not block initial binding of TPO to its receptor, had epitopes predominantly on helix or 3. These results suggest that TPO has two distinct receptor-binding sites that function to dimerize TPO receptors in a sequential fashion.

7/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09143754 97378100
Lipopolysaccharide and monophosphoryl lipid A differentially regulate interleukin-12, gamma interferon, and interleukin-10 mRNA production in murine macrophages.
Salkowski CA; Detore GR; Vogel SN
Department of Microbiology and Immunology, Uniformed Services, University of the Health Sciences, Bethesda, Maryland 20814, USA.
Infect Immun (UNITED STATES) Aug %1997%, 65 (8) p3239-47, ISSN 0019-9567 Journal Code: GO7
Contract/Grant No.: AI-18797, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Monophosphoryl lipid A (%MPL%) is a nontoxic derivative of the lipid A region of lipopolysaccharide (LPS) that is being developed as both an adjuvant and prophylactic drug for septic shock. We compared the ability of LPS and %MPL% to induce interleukin-10 (IL-10), IL-12 p35, IL-12 p40, gamma interferon (IFN-gamma), glucocorticoid receptor (GR), IL-1 receptor antagonist (IL-1ra), and inducible nitric oxide synthase mRNA expression in murine peritoneal macrophages. These genes were chosen for their ability to positively or negatively regulate the host immune response and thus for their potential involvement in %MPL% -induced adjuvanticity or in its ability to protect against sepsis. LPS was a more potent inducer of IL-12 p35, IL-12 p40, and IFN-gamma mRNA, as well as of IL-12 protein, than %MPL% . In contrast, %MPL% induced higher levels of IL-10 mRNA than did LPS from 1 to 1,000 ng/ml. In general, %MPL% was not a more potent inducer of negative regulatory genes, since %MPL% and LPS induced similar levels of GR and IL-1ra mRNA. Addition of anti-IL-10 %antibody% to cultures increased the induction of %MPL%-induced IL-12 p35, IL-12 p40, and IFN-gamma mRNA, suggesting that the enhanced production of IL-10 by %MPL%-stimulated macrophages contributes to decreased production of mRNA for IL-12 (p35 and p40)

and IFN-gamma. Conversely, the addition of exogenous IL-10 to LPS-treated macrophages reduced the mRNA expression of these cytokine genes. These studies suggest that enhanced production of IL-10 by %MPL% -stimulated macrophages may contribute to the reduced toxicity of %MPL% through its negative action on induction of cytokines shown to enhance endotoxicity.

7/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09124278 97304248
Adjuvants influence the quantitative and qualitative immune response in BALB/c mice immunized with respiratory syncytial virus FG subunit vaccine. Neuzil KM; Johnson JE; Tang YW; Prieels JP; Slaoui M; Gar N; Graham BS Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37232-2605, USA.
Vaccine (ENGLAND) Apr %1997%, 15 (5) p525-32, ISSN 0264-410X Journal Code: X60
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The ability of monophosphoryl lipid A (%MPL%), QS-21 and alum to alter the immunologic response to immunization with respiratory syncytial virus a chimeric FG construct (FG) subunit vaccine was examined in BALB/c mice. FG/ %MPL%, FG/alum, and FG/%MPL%/QS-21 combinations increased non-neutralizing %antibody% response, while FG/QS-21 did not. FG subunit vaccine with %MPL%, QS-21, or both had cytokine responses more closely resembling primary infection than FG/alum, with decreased interleukin-4 mRNA levels and increased IgG2a isotype %antibody%. The lungs of the mice immunized with FG subunit vaccines showed a heightened inflammatory response to respiratory syncytial virus challenge as compared to live virus immunization. Adjuvants can be used to alter the humoral and cellular responses to RSV subunit immunization.

7/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09050426 97269044
Characterization of the human thrombopoietin gene promoter. A possible role of an Ets transcription factor, E4TF1/GABP.
Kamura T; Handa H; Hamasaki N; Kitajima S
Department of Clinical Chemistry and Laboratory Medicine, Kyushu University, Faculty of Medicine, Fukuoka 812-82, Japan.
J Biol Chem (UNITED STATES) Apr 25 %1997%, 272 (17) p11361-8, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Thrombopoietin (TPO), the ligand for c-%Mpl% , is a cytokine that regulates megakaryocyte growth and development. We have cloned the 5'-flanking region of the human TPO gene and analyzed its promoter activity. The human TPO gene promoter lacks a TATA box and directs transcription initiation at multiple sites over a 50-nucleotide region. Transient expression in a human liver cell line (PLC) of promoter fragment-luciferase reporter gene constructs containing a series of 5'-truncated sequences or site-directed mutations identified a sequence 5'-ACTTCCG-3' from -69 to -63 as a positive cis-acting element for high level expression of TPO gene. This sequence contains a core motif (C/A)GGA(A/T) for Ets family proteins in the noncoding strand. Gel mobility shift assays performed with nuclear protein from PLC cells identified a DNA binding protein(s) specific for the element. Anti-E4TF1-60(GABPalpha) or anti-E4TF1-53/47(GABPbeta) %antibodies% supershifted the complex in gel shift assay. Furthermore, co-expression of E4TF1-60 and E4TF1-53/47 squelched TPO gene expression in PLC and HepG2 cells. It is concluded that Ets family transcription factor E4TF1(GABPalpha/beta), an ubiquitously expressed protein, is required for high level expression of the TPO gene in liver.

7/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08990974 97099321
Circulating thrombopoietin concentrations in thrombocytopenic patients, including cancer patients following chemotherapy, with or without peripheral blood progenitor cell transplantation.
Meng YG; Martin TG; Peterson ML; Shuman MA; Cohen RL; Wong WL BioAnalytical Technology and Molecular Oncology Department, Genentech Inc., South San Francisco, California, USA.
Br J Haematol (ENGLAND) Dec %1996%, 95 (3) p535-41, ISSN 0007-1048 Journal Code: AXC
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Thrombopoietin, the ligand for the c-%mpl% receptor, promotes proliferation and maturation of megakaryocytes. An ELISA using a chimaeric receptor, %mpl%-IgG, for capture, and rabbit %antibody% to thrombopoietin for detection was developed for the quantitation of thrombopoietin in human serum or

plasma. This ELISA preferentially detects full-length thrombopoietin compared to the bioactive N-terminal half of the molecule which has homology to erythropoietin. Thrombopoietin was not detected (< 0.16 ng/ml) in 88/89 healthy individuals. However, elevated thrombopoietin concentrations of up to 3 ng/ml were detected in 59/63 thrombocytopenic patients, including cancer patients following chemotherapy. In cancer patients receiving chemotherapy with (n = 12) or without (n = 6) peripheral blood progenitor cell transplantation, thrombopoietin concentrations varied inversely with platelet counts throughout the treatment period. In general, patients who received myeloablative chemotherapy on days -7 to -2 and peripheral blood progenitor cell transplantation on day 0 had high thrombopoietin levels (0.6-2.9 ng/ml) around day 5. Low platelet counts (< 20 x 10(9)/l) occurred between days 4 and 9. Patients who received high-dose chemotherapy on day 1 (equivalent to day -7 for transplantation patients) to day 6 without transplantation had high thrombopoietin concentrations (1.4-2.3 ng/ml) around day 13 and low platelet counts occurred between days 7 and 17.

7/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08990684 97194805
Direct and indirect stimulation of megakaryocytopoiesis by TAN-1511 A, a microbial lipopeptide.
Nishi K; Horiguchi T; Kawashima F; Tanida S
Discovery Research Laboratories II, Takeda Chemical Industries, Ltd, Ibaraki, Japan.
Anticancer Res (GREECE) Nov-Dec %1996%, 16 (6B) p3695-703, ISSN 0250-7005 Journal Code: 59L
Languages: ENGLISH
Document type: JOURNAL ARTICLE
TAN-1511 A, a microbial lipopeptide, stimulates granulocytopoiesis through the induction of various hematopoietic cytokines. The ability of synthetic TAN-1511 A to affect megakaryocytopoiesis was examined. TAN-1511. A augmented the activity of acetylcholine esterase (AChE), which is a cell-lineage marker enzyme of megakaryocytes in cultured murine bone marrow cells and enhanced megakaryocyte colony formation in fibrin clot culture. These effects were observed not only in the presence, but also in the absence of IL-3, which is a megakaryocyte colony-stimulating factor. The increase in AChE activity mediated by TAN-1511 A was blocked by anti-IL-6 but not anti-IL-3 or anti-GM-CSF neutralizing %antibodies%. RT-PCR analysis also showed that the remarkable induction of IL-6 was triggered by treatment with TAN-1511 A, while each message level of c-%mpl% ligand and c-%mpl% remained unchanged, suggesting that the IL-6 induced by the lipopeptide plays a role in enhanced megakaryocytopoiesis. TAN-1511 A also stimulated the proliferation of CMK86, a human megakaryoblastic cell line. This promoted growth was partially and additively affected by anti-IL-3, anti-IL-6, and anti-GM-CSF %antibodies%. TAN-1511 A slightly reduced the expression of GpIb and GpIIb/IL1a in CMK86 cells. The enhanced platelet recovery mediated by TAN-1511 A was also demonstrated in a model of myelosuppressive mice. These results suggest that TAN-1511 A directly affects megakaryocytopoiesis, and indirectly modulates megakaryocytopoiesis through the induction of cytokines such as IL-6.

7/3,AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08985354 97163916
Cytokine-containing liposomes as vaccine adjuvants.
Lachman LB; Ozpolat B; Rao XM
University of Texas M.D. Anderson Cancer Center, Department of Cell Biology, Houston 77030, USA.
Eur Cytokine Netw (FRANCE) Dec %1996%, 7 (4) p693-8, ISSN 1148-5493 Journal Code: A56
Contract/Grant No.: CAI 6672
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL The ability of cytokines to regulate and augment an immune response makes them likely agents to be included in vaccines. The systemic use of cytokines as vaccine adjuvants has been hampered by toxicity at effective doses. More precise delivery of cytokines and immunogen can be achieved through the use of microparticle carriers such as liposomes. Several cytokines, including IL-2, IL-7, IL-6 and IFN-gamma have been shown to increase the adjuvant activity of liposomes. It may be possible to use certain cytokines to induce immunoglobulin production, others to induce cytotoxic T lymphocytes (CTL) and still others to promote IgA production at mucosal surfaces. An alternative to liposomes containing cytokines may be liposomes containing cytokine-inducing adjuvants such as MTPPE, %MPL% and QS21. This approach may produce undesirable immunomodulators as well as beneficial cytokines.

7/3,AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08980624 97124948
VH4-34 (VH4.21) gene expression in the chronic

arthritides of childhood: studies of associations with anti-lipid A %antibodies%, HLA antigens, and clinical features.
Miller JJ 3rd; Bieber MM; Levinson JE; Zhu S; Tsou E; Teng NN Department of Pediatrics, Stanford University School of Medicine, California, USA.
J Rheumatol (CANADA) Dec %1996%, 23 (12) p2132-9, ISSN 0315-162X Journal Code: JWX
Contract/Grant No.: AR 42632, AR, NIAMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
OBJECTIVE: To determine if the germ line gene VH4-34 (VH4.21) encodes the antimonophosphoryl lipid A (%MPL%) polyspecific %antibodies% found in oligoarticular arthritis of childhood. METHODS: Sera from a range of rheumatic diseases of childhood were assayed for VH4-34 derived %antibodies% by ELISA using the antiidiotype monoclonal %antibody% 9G4. Results were compared to assays for anti-%MPL% %antibodies%, C4d, and Bb, and for HLA type, joint count, and sedimentation rate. RESULTS: VH4-34 derived %antibodies% were elevated in all diseases studied except rheumatoid factor positive polyarticular disease. In oligoarticular arthritis, VH4-34 gene expression correlated with C4d concentration, and VH4-34 encoded globulins were more concentrated in synovial fluid than in blood. No association was found with HLA type. An association between VH4-34 expression and IgG anti-%MPL% was found in sera from patients from Cincinnati but not from Stanford. No other evidence supported a direct association between VH4-34 derived and anti-%MPL% %antibodies% in these children. CONCLUSION: The expression of VH4-34 is increased in several rheumatic diseases of childhood, but, as in adults, not in rheumatoid arthritis. VH4-34 expression is not associated with HLA type. The polyspecific autoantibody nature of some VH4-34 derived %antibodies% may explain the wide range of the unusual %antibodies% found in oligoarticular arthritis.

7/3,AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08975358 97109490
Clonality assays and megakaryocyte culture techniques in essential thrombocythemia.
Yan L; Elkassar N; Gardin C; Briere J
Haematology Division, Hospital Beaujon, Clichy, France.
Leuk Lymphoma (SWITZERLAND) Sep %1996%, 22 Suppl 1 p31-40, ISSN 1042-8194 Journal Code: BNQ
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL The development of techniques permitting in vitro growth of human megakaryocytes progenitors and more recently identification of the proto oncogene c-%mpl% (%Mpl%-R) and its ligand (%Mpl% -L) have created new opportunities for studying pathophysiology of E.T. Plasma or serum of E.T. patients was unable to overestimulate MK colony formation by normal bone marrow cells. Significant increases in circulating CFU MK in E.T. patients have been repeatedly observed while in E.T. marrow, due to inappropriate sampling, colony number was not significantly different from normal. Spontaneous colony formation is observed in approximately 100% bone marrow and 85% blood from E.T. patients. Spontaneous colony formation persisted in plasma clot assay without added plasma or serum and in serum free agar cultures but only at a slightly lower rate than in plasma clot. Spontaneous colony formation in culture condition without plasma and serum were never observed with normal bone marrow and blood. Spontaneous MK growth was observed in a higher proportion of E.T. patients than erythroid colony formation but both phenomenon can occur in about 50% of the patients. CFU MK colony formation disappeared in serum free cultures using highly purified CD 34 cells. MK development is not completely independent of regular control. An hypersensitivity of E.T. MK progenitors to growth factors known to stimulate normal hematopoiesis (IL3.IL6, GM CSF, has been shown as well as a decreased sensitivity to negative regulators (TGF beta), has been suggested. The number of spontaneous MK colonies was not significantly decreased by added anti IL3, IL6 or anti GM CSF, %antibodies% in culture medium. Pre incubation of blood non adherent mononuclear cells of E.T. patients with antisense oligonucleotides to c-%mpl% significantly decreased the cloning efficiency of spontaneous megakaryocyte growth as compared to the introduction of scrambled oligomers. Finally m RNA expression of the %Mpl%-L (TPO) was not formed in MK spontaneously grown in serum free liquid cultures after 12 days. These results suggest that human c-%mpl% proto oncogene may be implicated in the pathway of spontaneous megakaryocytopoiesis in MPD but an absence of autocrine-stimulation by TPO of spontaneous growth in MPD. Analysis of peripheral blood cell clonality was performed in 55 E.T. patients using either the DNA methylation pattern of the androgen receptor (AR) gene or mRNA transcripts of G6PD or IDS genes. 51 out of 55 patients were informative. Non random X inactivation was found on unfractioned blood in 73% as compared with 23% in normal females (skewed Lyonisation). In 12 patients monoclonality of hematopoiesis was definitely confirmed by recording polyclonality of the mononuclear fraction or of T lymphocytes. In 4 patients monoclonal hematopoiesis was limited to platelets, 7 patients remained polyclonal in whole blood and all cellular fractions studied. MK colony formation (provided that the serum free agar culture

system is clearly standardised) and clonality studies on whole blood or granulocyte, T lymphocyte and platelet fractions may be proposed as positive criteria for diagnosis of E.T.

7/3,AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08972018 97127161
Lupus anticoagulant is the strongest risk factor for both venous and arterial thrombosis in patients with systemic lupus erythematosus. Comparison between different assays for the detection of antiphospholipid %antibodies% [see comments]
Horbach DA; van Oort E; Donders RC; Derksen RH; de Groot PG Department of Haematology, University Hospital Utrecht, The Netherlands. Thromb Haemost (GERMANY) Dec %1996%, 76 (6) p916-24, ISSN 0340-6245 Journal Code: VQ7
Comment in Thromb Haemost 1997 Aug;78(2):967-8
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Antiphospholipid %antibodies% (aPL) characterize patients at risk for both arterial and venous thrombotic complications. Recently it has been recognized that the presence of plasma proteins such as beta 2-glycoprotein I(beta 2 GPI) and prothrombin are essential for the binding of aPL to phospholipids and that these proteins are probably the real target of aPL. The discovery of these new antigens for aPL introduces the possibility of new assays to detect the presence of aPL. However, it is not known whether these assays improve the identification of patients at risk for thrombosis. In this retrospective study we compared the value of the classic assays LAC (lupus anticoagulant) and ACA (anticardiolipin %antibodies%) to detect aPL associated with thrombotic complications, with new assays which are based on the binding of aPL to the plasma proteins prothrombin and beta 2GPI. To do so, we have used these assays in a group of 175 SLE patients and correlated the positivity of the different assays with the presence of a history of venous and arterial thrombosis. Control groups were patients without SLE but with LAC and/or ACA and thrombosis (n = 23), patients with thrombosis without LAC and ACA (n = 40) and 42 healthy controls. In the univariate analysis, in which no distinction has been made between high and low %antibody% levels, we confirmed LAC and ACA to be related to both arterial and venous thrombosis. Anti-beta 2GPI- and anti-prothrombin- %antibodies% , both IgG and IgM correlate with venous thrombosis and anti-beta 2GPI-IgM with arterial thrombosis. Multivariate analysis showed that LAC is the strongest risk factor (OR 9.77; 95% CI 1.74-31.15) for arterial thrombosis. None of the other factors is a significant additional risk factor. For venous thrombosis LAC is the strongest risk factor (OR 6.55; 95% CI 2.36-18.17), but ACA-IgM above 20 %MPL% units also appeared to be a significant (p = 0.0159) risk factor (OR 3.90; 95% CI 1.29-11.80). Furthermore, the presence of anti-beta 2GPI- and/or anti-prothrombin- %antibodies% in LAC positive patients (n = 60) does not increase the risk for thrombosis. The results showed that (i) the LAC assay correlates best with a history of both arterial and venous thrombosis and (ii) neither the anti-beta 2GPI ELISA nor the anti-prothrombin ELISA gives additional information for a thrombotic risk in SLE patients.

7/3,AB/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08963427 96426563
Identification, characterization, and expression of a new prolactin-like molecule in the hamster placenta.
Barnes SW; Renegar RH
Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, North Carolina 27858, USA.
Biol Reprod (UNITED STATES) Aug %1996%, 55 (2) p370-8, ISSN 0006-3363 Journal Code: A3W
Contract/Grant No.: HD-23481, HD, NICHD
Languages: ENGLISH
Document type: JOURNAL ARTICLE
In the hamster, serum total lactogenic activity increases during the latter half of gestation (Days 8-16). On Days 10 and 12 a substantial amount of lactogenic activity cannot be attributed to prolactin (PRL) and hamster placental lactogen-II (haPL-II); therefore, the presence of a molecule similar to placental lactogen-I (PL-I), as found in the rat and mouse at midpregnancy, has been hypothesized for the hamster. The objectives of this study were to identify PRL-like molecules synthesized by the hamster placenta and to determine the temporal and cellular synthesis of identified molecules. Oligonucleotides (20-23 bp) corresponding to regions of nucleotide homology between mouse PL-I (%mPL%-I) and rat PL-I (rPL-I) along with midgestation hamster placental RNA were used in 3' rapid amplification of cDNA ends (RACE) methodology to generate PRL-like cDNA. A 444-bp cDNA fragment that had nucleotide sequence similarity with members of the prolactin-growth hormone (PRL-GH) gene family was generated. This cDNA fragment was utilized to screen a Day 16 hamster placental bacteriophage cDNA library, and a clone containing the entire coding region was identified and sequenced. The molecule had 77% nucleotide sequence homology with mouse

proliferin-related protein (mPRP) and somewhat less homology (approximately 60%) with hamster, rat, and mouse PRL or placental lactogens (PL). The derived amino acid sequence of the identified molecule contained a 15-residue signal sequence and a 219-residue peptide with a calculated molecular weight of 25477. The peptide shared 58% amino acid sequence identity with mPRP. Placental expression of the PRL-like molecule during the latter half of gestation was evaluated by Northern and slot-blot analyses using the 444-bp cDNA fragment as a hybridization probe. A 1-kb transcript was detected on Days 9-15 with peak expression on Days 10 and 11. Messenger RNA for the PRL-like molecule was localized to cytotrophoblast but not giant trophoblast cells of the placental trophospongium region. In addition, specific immunostaining using an %antibody% to mPRP was confined to cytotrophoblast cells.

7/3,AB/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08921303 97038953
Lipopolysaccharides selectively inhibit mouse placental lactogen-II secretion through stimulation of interleukin-1 alpha (IL-1 alpha) and IL-6 production.
Yamaguchi M; Sawada K; Miyake A
Osaka University Medical School, Japan.
J Endocrinol Invest (ITALY) Jul-Aug %1996%, 19 (7) p415-21, ISSN 0391-4097 Journal Code: IAM
Languages: ENGLISH
Document type: JOURNAL ARTICLE
To determine whether lipopolysaccharides (LPS) regulate mouse placental lactogen-I (%mPL%-I), %mPL% -II, and mouse GHRF (mGHRF) secretion, mouse placental tissue from days 7, 9, and 12 of pregnancy was dispersed with collagenase and the purified trophoblast cells were cultured in a serum-free medium with or without LPS for 5 days. LPS significantly inhibited %mPL% -II secretion by cells from days 9 and 12 of pregnancy. However, LPS did not affect %mPL% -II secretion by cells from day 7 of pregnancy, %mPL% -I secretion by cells from days 7 and 9 of pregnancy, or mGHRF secretion by cells from day 12 of pregnancy. The inhibitory effect of LPS on %mPL% -II secretion by cells from day 12 of pregnancy was dose-dependent. Steady-state levels of %mPL%-II mRNA were significantly reduced by incubation of placental cells from day 12 of pregnancy with LPS. The inhibitory effect of LPS on %mPL%-II secretion was abolished by the addition of %antibodies% to IL-1 alpha and IL-6. These findings suggest that LPS selectively inhibit %mPL%-II secretion, at least partly through increases in IL-1 and IL-6 production, after midpregnancy.

7/3,AB/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08920936 97037087
Effects of monophosphoryllipid-A on the immunization of mice with keyhole limpet hemocyanin- and muramyldipeptide-ganglioside Gfpt1 conjugates.
Jennemann R; Bauer BL; Schmidt R; Elsasser HP; Wiegandt H Abtlg. Neurochirurgie, Philipps-Universitat, Marburg, Germany. J Biochem (Tokyo) (JAPAN) Feb %1996%, 119 (2) p378-84, ISSN 0021-924X Journal Code: HIF
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Since it was considered that an active immunization against ganglioside Gfpt1 (IV2Fuc-, II3NeuAc-Gg4Cer) expressed by human small cell lung cancer cells may be beneficial in the treatment of this neoplasm in humans, an optimal mode of vaccination in model mice was investigated. A novel Gfpt1-muramyldipeptide conjugate (Gfpt1-MDP) was synthesized. Its ganglioside carbohydrate-directed immunogenicity in mice as measured by serum %antibody% titers was comparable to that of the previously described Gfpt1-keyhole limpet hemocyanin conjugate (Gfpt1-KLH). Similar immunogenicity was displayed by free Gfpt1 in muramyldipeptide-phosphoethan olamine-containing phosphatidyl-choline, -serine (PC,PS) liposomes. Immunization with Gfpt1-vaccines in the presence of monophosphoryllipid A (%MPL%), in general, raised titers of anti-Gfpt1 %antibodies% effectively. Immunization with PC, PS-liposomes containing unconjugated Gfpt1 and %MPL% stimulated the highest titers observed, thereby effectively preventing tumor growth in Balbc nu/nu-mice challenged with human small cell lung cancer cells. However, there was a strong crossreaction of these and most other sera with the structurally related and widely distributed ganglioside Gtet1 (II3NeuAc-Gg4Cer). Only immunization with Gfpt1-KLH conjugate in the presence of %MPL% stimulated selectively high anti-Gfpt1 %antibody% titers showing comparably low crossreactivity to ganglioside Gtet1.

7/3,AB/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08908423 96231437
A new approach to labeling cells with technetium-99m, Part II. Evaluation of radiolabeled human-lymphocyte immune functions.
Delmon-Moingeon LI; Mahmood A; Davison A; Jones AG
Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA USA.

Nucl Med Biol (ENGLAND) Jan %1996%, 23 (1) p87-95, ISSN 0969-8051 Journal Code: BOO
Contract/Grant No.: 5 R37 CA34970, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Technetium-99m-modified polylysine (%MPL%) is a mild and efficient method for cell labeling that is easily applicable to human lymphocytes. 99mTc- %MPL% uptake is maximal in 40 min at room temperature. Cell labeling efficiency (from 60 to 80%) increases with rising concentrations of cells and labeling agent. In vitro stability of 99mTc-%MPL% on lymphocytes incubated in serum at 37 degrees C is high. Microautoradiography indicates that 99mTc-%MPL% is equally distributed within the cells. The radiolabeling procedure does not alter expression of surface receptors involved in T lymphocyte effector functions, adhesion function, cytolytic activity or proliferative response to IL-2.

7/3,AB/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08867312 97099290
Crkl is constitutively tyrosine phosphorylated in platelets from chronic myelogenous leukemia patients and inducibly phosphorylated in normal platelets stimulated by thrombopoietin.
Oda A; Miyakawa Y; Druker BJ; Ishida A; Ozaki K; Ohashi H; Wakui M; Handa M; Watanabe K; Okamoto S; Ikeda Y
Department of Internal Medicine, Keio University, Tokyo, Japan. Blood (UNITED STATES) Dec 1 %1996%, 88 (11) p4304-13, ISSN 0006-4971 Journal Code: A8G
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Platelet functions such as aggregation and clot retraction are often abnormal in chronic mylogenous leukemia (CML) patients. However, the molecular mechanisms of these altered functions are unknown. As expression of the p210bcr-abl oncogene product, a constitutively active tyrosine kinase, is known to have an essential role in the pathogenesis of CML and tyrosine phosphorylation is intimately involved in various aspects of platelet activation, we examined the pattern of protein tyrosine phosphorylation in platelets from 15 CML patients by immunoblotting with a monoclonal antiphosphotyrosine %antibody% (4G10). Before and after stimulation with thrombin, the only consistent difference between normal and CML platelets was the presence of a tyrosine phosphorylated protein with a relative molecular weight of 39 kD. This tyrosine phosphorylated protein was identified as crid, an SH2, SH3 containing adapter protein. Thus, as previously demonstrated for neutrophils from CML patients, tyrosine phosphorylation of p39crkl persists in mature platelets. No tyrosine phosphorylation of crid was detected following stimulation with thrombin in normal platelets. However, crkl became incorporated into the Triton X-100 insoluble residue following thrombin stimulation in a manner dependent on platelet aggregation. Further, we found that crkl is an endogenous substrate for calpain, a protease that may be involved in postaggregation signaling processes. This suggests that crkl may be involved in the reorganization of the cytoskeleton during normal platelet aggregation and its tyrosine phosphorylation in CML platelets may contribute to the abnormal platelet function in CML patients. Finally, we found that thrombopoietin induces tyrosine phosphorylation of crk1 in normal platelets and FDCP cells genetically engineered to express human c- %Mpl%. This suggests that crk1 can be phosphorylated by a kinase other than p210bcr-abl and that crk1 may have a role in signaling by thrombopoietin.

7/3,AB/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08774101 96420222
Thrombopoietin stimulates colony-forming unit-megakaryocyte proliferation and megakaryocyte maturation independently of cytokines that signal through the gp130 receptor subunit.
Broudy VC; Lin NL; Fox N; Taga T; Saito M; Kaushansky K
University of Washington, Division of Hematology, Seattle 98195-7710, USA.
Blood (UNITED STATES) Sep 15 %1996%, 88 (6) p2026-32, ISSN 0006-4971 Journal Code: A8G
Contract/Grant No.: DK 44194, DK, NIDDK; CA 31615, CA, NCI; DK 49855, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Thrombopoietin (Tpo), the ligand for the c-%Mpl% receptor, is a major regulator of megakaryopoiesis. Treatment of mice with Tpo raises the platelet count fourfold within a few days. Conversely, c-%mpl% knock-out mice have platelet counts that are 15% that of normal. The subunit structure of the c-%Mpl% receptor is not fully understood. Some cytokines that stimulate megakaryopoiesis (IL-6, IL-11, leukemia inhibitory factor, and oncostatin M) bind to receptors that use gp130 as a signal transduction subunit. For these reasons, we determined whether gp130 function was required for Tpo-induced signal transduction. Murine marrow cells were cultured in semi-solid media in the presence of Tpo or IL-3, with or without a neutralizing anti-gp130 monoclonal %antibody% (RX187) or a soluble form of c-%Mpl% receptor (soluble %Mpl%) that blocks Tpo bioactivity, and the numbers

of colony-forming unit-megakaryocyte (CFU-Meg) colonies were counted on day 5. Murine marrow cells were also cultured in suspension under serum-free conditions for 5 days, and megakaryocyte DNA content was measured by flow cytometry, as an index of nuclear maturation. The addition of RX187 did not block Tpo-induced CFU-Meg colony growth nor CFU-Meg nuclear maturation in suspension culture. However, IL-3-induced CFU-Meg colony growth and megakaryocyte nuclear maturation decreased in the presence of RX187. Soluble %Mpl% completely ablated Tpo-induced CFU-Meg growth, and partially blocked IL-3-stimulated CFU-Meg growth. Thus the effects of Tpo on megakaryopoiesis in vitro do not depend on cytokines that signal through gp130. Furthermore, it is unlikely that gp 130 serves as a beta chain for the c-%Mpl% receptor, as Tpo signalling is unimpaired in the presence of RX187. In contrast, the effects of IL-3 on CFU-Meg growth are mediated in part through Tpo and through gp130-signalling cytokines.

7/3,AB/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08731500 96313014
Safety, immunogenicity, and efficacy of Plasmodium falciparum repeatless circumsporozoite protein vaccine encapsulated in liposomes. Heppner DG; Gordon DM; Gross M; Wellde B; Leitner W; Krzych U; Schneider I; Wirtz RA; Richards RL; Trofa A; Hall T; Sadoff JC; Boerger P; Alving CR; Sylvester DR; Porter TG; Ballou WR
Department of Membrane Biochemistry, Walter Reed Army Institute of Research, Washington, DC, USA.
J Infect Dis (UNITED STATES) Aug %1996%, 174 (2) p361-6, ISSN 0022-1899 Journal Code: IH3
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Seventeen malaria-naive volunteers received a recombinant Plasmodium falciparum vaccine (RLF) containing the carboxy- and the amino-terminal of the circumsporozoite protein (CSP) antigen without the central tetrapeptide repeats. The vaccine was formulated in liposomes with either a low or high dose of 3-deacylated monophosphoryl lipid A (%MPL%) and administered with alum by intramuscular injection. Both formulations were well tolerated and immunogenic. %MPL% increased sporozoite %antibody% titers measured by ELISA, Western blot, and immunofluorescence assay. One high-dose %MPL% vaccine formulation recipient developed a CSP-specific cytotoxic T lymphocyte response. After homologous sporozoite challenge, immunized volunteers developed patent malaria. There was no correlation between prepatent period and %antibody% titers to the amino- or carboxy-terminal. The absence of delay in patency argues against inclusion of the amino-terminal in future vaccines. A significant cytotoxic T lymphocyte response may have been suppressed by the inclusion of alum as an adjuvant.

7/3,AB/33 (Item 33 from file: 155)
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08719718 96236818
Distribution and localization of monophosphoryl lipid A in selected tissues of the rat.
Reddy TS; Kishore V
Xavier University of Louisiana, College of Pharmacy, New Orleans 70125, USA.
Immunopharmacol Immunotoxicol (UNITED STATES) Feb %1996%, 18 (1) p145-59, ISSN 0892-3973 Journal Code: IAI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The distribution and cellular localization of Monophosphoryl Lipid A in the kidney, liver, lung, spleen, and stomach of rat were investigated by immunohistochemistry on paraffin embedded tissue sections. Rats were sacrificed 24h or 48h following intraperitoneal administration of %MPL% at a dose of 5mg/Kg. The presence of %MPL% in selected tissues was indicated by a positive reaction to %MPL% %antibody%. In the kidneys, %MPL% was found in collecting tubules and distal convoluted tubules in the medulla, whereas the glomerulus was essentially free of it. Regarding liver, %MPL% was found to be abundant in hepatocytes but only occasionally present in Kupffer cells. In lungs, both alveolar and bronchiolar macrophages were positive, indicating the lung can also serve as a possible site for the elimination of %MPL%. In spleen, endothelial cells and macrophages were positive for %MPL%. In stomach, %MPL% was detected in the gastric mucosa and vascular endothelial cells. In all the tissues studied the intensity of the peroxidase reaction was significantly weaker in 48h samples as compared to corresponding 24h samples indicating possible elimination of %MPL% from these tissues.

7/3,AB/34 (Item 34 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08693771 96219640
Characterization of vasoactive intestinal peptide receptors on human megakaryocytes and platelets.
Park SK; Olson TA; Ercal N; Summers M; O'Dorisio MS
Department of Pediatrics, The Ohio State University, Columbus, USA. Blood (UNITED STATES) Jun 1 %1996%, 87 (11) p4629-35, ISSN 0006-4971 Journal Code: ABG

Contract/Grant No.: CA29062, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Vasoactive intestinal peptide receptor I (VIPRI) expression was examined in megakaryocytes using reverse transcriptase-polymerase chain reaction (RT-PCR). VIPRI protein was characterized in platelet membranes using covalent crosslinking techniques. Human megakaryocytes were isolated from suspension cultures of cord blood and adult bone marrow mononuclear cells using a murine monoclonal %antibody% to human platelet glycoprotein IIB/IIIA (CD41) and immunomagnetic beads. RT-PCR primers were constructed for the VIP, VIPRI, and VIPRII genes as well as for megakaryocyte specific genes, c-%mpl% and platelet factor 4 (PF-4). VIP, VIPRI, c-%mpl%, and PF-4 were coexpressed in megakaryocyte mRNA. Southern blot analysis confirmed the expression of VIPRI. 125I-VIP was covalently cross-linked to human platelet membranes using the homobifunctional reagent disuccinimidyl suberate, followed by polyacrylamide gel electrophoresis and autoradiography. A 125I-VIP-protein complex of Mr = 50,000 was identified. Labeling of the Mr = 50,000 component was completely abolished by unlabeled VIP, but not by peptide histidine methionine or growth hormone releasing factor, indicating specific binding of VIP to the platelet membranes. Taken together, these results suggest that VIP may have direct effects on megakaryocytopoiesis and support our earlier observations of VIP modulation of platelet aggregation.

7/3,AB/35 (Item 35 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08693764 96219631
Thrombopoietin, the ligand for the %Mpl% receptor, synergizes with steel factor and other early acting cytokines in supporting proliferation of primitive hematopoietic progenitors of mice.
Ku H; Yonemura Y; Kaushansky K; Ogawa M
Department of Medicine, Medical University of South Carolina, Charleston, USA.
Blood (UNITED STATES) Jun 1 %1996%, 87 (11) p4544-51, ISSN 0006-4971 Journal Code: A8G
Contract/Grant No.: DK 32294, DK, NIDDK; DK 48714, DK, NIDDK; DK 49855, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Recently, the ligand for the %Mpl% receptor (ML) was identified to be thrombopoietin, the principal regulator of megakaryocytopoiesis and thrombopoiesis. We examined the effects of ML, as a single factor or in combinations with early acting factors such as steel factor (SF), interleukin (IL)-3, IL-1, IL-6, and granulocyte colony-stimulating factor (G-CSF), on colony formation from primitive progenitors of mice. Cells enriched for cell cycle dormant primitive progenitors were isolated from bone marrow cells of 5-fluorouracil (5-FU)-treated mice by a combination of Nycodenz density gradient separation, immunomagnetic selection for lineage-negative cells, and fluorescence-activated cell sorter (FACS) sorting for Ly-6A/E+Kit+ cells. ML, in the presence of erythropoietin, could support the formation of only a few megakaryocyte colonies. However, ML acted synergistically with SF or IL-3 to support the formation of multiple types of hematopoietic colonies including multilineage colonies. Effects of the combination of ML and SF on multipotential progenitors were not mediated through other cells, as demonstrated by micromanipulation of individual progenitors. In suspension culture, the combination of ML and SF increased the number of multipotential progenitors. ML also acted synergistically with IL-11, IL-6, or G-CSF to support colony formation in serum-containing, but not in serum-free, cultures. However, the multilineage colony formation seen in serum-containing culture was completely abrogated by addition of ACK2, a neutralizing %antibody% to Kit protein. Serial observation (mapping studies) of colony development from multipotential progenitors suggested that ML triggers the cell division of dormant progenitors. Based on these observations, we propose that ML can function as an early acting cytokine and stimulate the proliferation of cell cycle dormant progenitors by shortening their G0 period.

7/3,AB/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08671278 96185050
Insights into the cellular mechanisms of erythropoietin-thrombopoietin synergy.
Papayannopoulou T; Brice M; Farrer D; Kaushansky K
Department of Medicine, University of Washington, Seattle, USA. Exp Hematol (UNITED STATES) Apr %1996%, 24 (5) p660-9, ISSN 0301-472X Journal Code: EPR
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Using suspension cultures of purified bone marrow CD34+ cells, we have analyzed the effects of the combination of erythropoietin (Epo) and thrombopoietin (Tpo) on the in vitro differentiation toward erythropoiesis and thrombopoiesis. The number of CD41+ cells that accumulated over 2 weeks of culture, as well as the number of globin+ cells in the same cultures, was found to be significantly higher with the Epo+Tpo combination compared to either cytokine alone. No evidence was

found that Tpo affected the differentiative action of Epo. Instead, there was a significant expansion of erythroid progenitors, both erythroid colony-forming and burst-forming units (CRU-E and BFU-E), by 7 days in culture, suggesting a proliferative effect of Tpo on erythroid cells in vitro. To determine the phenotypic features of erythroid progenitor cells which were targets of Tpo's action, and specifically to inquire whether the effect was directed mainly toward bipotent erythroid/megakaryocytic (E+Mk) progenitors, we isolated subsets enriched for both erythroid and megakaryocytic progenitors from CD34+ cells. We found that 1) BFU-E and CFU-Mk co-segregate in the subset of CF34+ cells that is negative for the phosphatase isoform CD45RA; 2) the presence of CD41 on this subset appears to segregate late erythroid and late CFU-Mk from early erythroid and early CFU-Mk, which are CD41-negative; 3) bipotent erythroid/Mk progenitors, studied by single-cell culture assays, were found mainly in the CD41+ and rarely in the CD41- subsets which included more multipotent progenitors; 4) by comparing the frequencies of pure erythroid or pure megakaryocytic progenitors to that of bipotent E+Mk progenitors, we conclude that the erythroid-enhancing effect of Tpo is directed mainly toward pure erythroid progenitors expressing CD41 and %Mpl%, as suggested by independent experiments employing anti-%Mpl% %antibody%, rather than only on bipotent E+Mk progenitors.

7/3,AB/37 (Item 37 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08660516 96136037
Proto-oncogene c-%mpl% is involved in spontaneous megakaryocytopoiesis in myeloproliferative disorders.
Li Y; Hetet G; Kiladjian JJ; Gardin C; Grandchamp B; Briere J Genetique et Pathologie Moeculaires de l'Hematopoies, INSERM U409, Association Claude Bernard, Clichy, France.
Br J Haematol (ENGLAND) Jan %1996%, 92 (1) p60-6, ISSN 0007-1048 Journal Code: AXC
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Spontaneous megakaryocytic colonies (CFU-MK) formation without the addition of Meg-CSA in myeloproliferative disorders (MPD) has been reported by many laboratories. The mechanism by which this occurs is still unknown. In our previous work we have found that the spontaneous colonies persisted in serum-free agar culture although the colony cells were smaller and the colony numbers fewer than in plasma clot culture and that monoclonal %antibodies% against IL3, IL6 and GM-CSF had no inhibitory effect on spontaneous CFU-MK in both semisolid cultures. Recently, proto-oncogene c- %mpl% and c-%mpl% ligand, thrombopoietin (TPO), have been shown to specifically participate in the regulation of normal human megakaryocytopoiesis. In order to test the hypothesis that c-%mpl%, c-%mpl% ligand pathway is involved in the spontaneous growth of megakaryocyte progenitors, we investigated mRNA expressions of c-%mpl% and TPO in cells grown in serum-free liquid culture using RT-PCR. The c-%mpl% expression was detected in the cultured cells from all nine patients (six with ET, two with PV, one with PMF) who had spontaneous CFU-MK in clonal assays. However, none of the patients expressed TPO mRNA in these cells. Pre-incubation of nonadherent mononuclear cells with thioester-modified antisense oligodeoxynucleotide to c-%mpl% at a concentration of 6 microM significantly decreased the cloning efficiency of spontaneous megakaryocyte growth by 42.5% ($P < 0.05$) in plasma clot assay (seven with ET, one with PV) and 69.6% ($P < 0.05$) in serum-free agar culture (six with ET, one with PV). In control experiments the introduction of a scrambled oligomer to antisense oligodeoxynucleotide had no such effect on spontaneous colony formation. These results indicate that c-%mpl% exerts an important effect in the growth of spontaneous megakaryocytopoiesis in MPD.

7/3,AB/38 (Item 38 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08657352 96145078
Variation of Brucella abortus 2308 infection in BALB/c mice induced by prior vaccination with salt-extractable periplasmic proteins from Brucella abortus 19.
Pugh GW Jr; Tabatabai LB
National Animal Disease Center, United State Department of Agriculture, Ames, Iowa, USA.
Infect Immun (UNITED STATES) Feb %1996%, 64 (2) p548-56, ISSN 0019-9567 Journal Code: GO7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The study compared the immune and protective responses induced in BALB/c mice vaccinated with six salt-extractable periplasmic protein fractions (Brucella cell surface proteins [BCSP]) of Brucella abortus 19 and later challenge exposed with B. abortus 2308. BCSP70 was precipitated with ammonium sulfate at 70% saturation, and BCSP100 was precipitated with ammonium sulfate at 100% saturation by use of supernatant fluid of BCSP70 that had been precipitated with 70% ammonium sulfate. Four subfractions were separated from BCSP100 by anion-exchange high-performance liquid chromatography (HPLC). Monophosphoryl lipid A (%MPL%) from Salmonella typhimurium Re mutant strain was used as a potential

immune response modifier in some vaccines. Reduced or increased numbers of CFU and increased spleen size in the principal groups of mice relative to that of the nonvaccinated control group were considered protectiveness or virulence (survival) criteria. Results indicated that vaccines prepared from BCSP70 and BCSP100 were moderately protective and immunogenic. The subfractions designated BCSP100-A through BCSP100-D purified by anion-exchange HPLC were not protective when %MPL% was not used as an immune response modifier. However, two subfractions were associated with significant ($P < 0.05$) increases in CFU per spleen and splenomegaly in vaccinated mice compared with those in nonvaccinated challenge-exposed mice. %MPL% enhanced protection or was neutral when used with BCSP70, BCSP100, BCSP100-C, and BCSP100-D. Serologic results of an enzyme-linked immunosorbent assay indicated that %MPL% modulated the immunoglobulin G responses induced by BCSP70, BCSP100, and subfraction BCSP100-B vaccines only. The overall results suggest that certain proteinaceous periplasmic fractions might serve as virulence or survival factors in B. abortus infections.

7/3,AB/39 (Item 39 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08631818 96115014
Cytokines that use gp130 as a signal transducer stimulate mouse placental lactogen-I (%mPL%-I) but inhibit %mPL%-II production in vitro. Yamaguchi M; Taga T; Kishimoto T; Miyake A
Department of Obstetrics and Gynecology, Osaka University Medical School, Japan.
Biol Reprod (UNITED STATES) Aug %1995%, 53 (2) p399-406, ISSN 0006-3363 Journal Code: A3W
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Interleukin-11 (IL-11), leukemia inhibitory factor (LIF), and oncostatin M (OM), all of which use gp130 as a signal transducer, significantly inhibited mouse placental lactogen-II (%mPL% -II) secretion by cultured placental cells from Days 7, 9, and 12 of pregnancy. These cytokines significantly stimulated %mPL%-I secretion by cells from Day 9, but not Day 7, of pregnancy. An %antibody% to LIF completely blocked the stimulatory and inhibitory effects of LIF on %mPL%-I and %mPL% -II secretion, respectively. LIF and OM decreased the abundance of %mPL%-II mRNA in placental cells. Double immunocytochemistry for %mPL%-I and %mPL%-II indicated that LIF, OM, and IL-11 significantly increased the number of giant cells containing only %mPL%-I or both %mPL%-I and %mPL%-II but decreased the number of giant cells containing only %mPL%-II. IL-6, which also uses gp130 as a signal transducer, inhibits %mPL%-II secretion only after midpregnancy; however, addition of soluble IL-6 receptor (sIL-6R) together with IL-6 resulted in a significant inhibition of %mPL%-II secretion before midpregnancy. Treatment of cells from Day 12 of pregnancy with IL-6 during the first 2 days of culture resulted in significant inhibition of %mPL% -II secretion by the third day of culture.(ABSTRACT TRUNCATED AT 250 WORDS)

7/3,AB/40 (Item 40 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08629427 96082158
Regulation of platelet activation in vitro by the c-%Mpl% ligand, thrombopoietin.
Chen J; Herceg-Harjacek L; Groopman JE; Grabarek J
Department of Medicine, Deaconess Hospital, Boston, MA 02215, USA. Blood (UNITED STATES) Dec 1 %1995%, 86 (11) p4054-62, ISSN 0006-4971 Journal Code: A8G
Contract/Grant No.: HL50562, HL, NHLBI; HL53745, HL, NHLBI; HL55187, HL, NHLBI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Thrombopoietin (TPO) is a recently identified growth factor that regulates megakaryocytopoiesis. Its receptor, c-%Mpl% , is expressed in megakaryocyte progenitors, mature megakaryocytes, and human blood platelets. We have observed that TPO treatment of human platelets resulted in tyrosine phosphorylation of several cellular proteins, including the c- %Mpl% receptor and the 85-kD subunit of phosphatidylinositol 3-kinase (PI3-K). TPO stimulated this tyrosine phosphorylation in a time-dependent manner, reaching a maximum in 5 minutes. The tyrosine phosphorylation of PI 3-K was dependent on the concentration of TPO and reached a maximum at concentrations between 50 and 100 ng/mL. This phosphorylation was independent of extracellular fibrinogen and ligation of the alpha IIb beta 3 integrin. In contrast, TPO, in the presence of exogenous fibrinogen, induced concentration-dependent platelet aggregation, which was blocked by the soluble c-%Mpl% receptor. Increasing TPO concentrations modulated the degree of the primary wave of aggregation and the lag phase, but not the slope or maximum of the secondary wave of aggregation. This secondary aggregation was controlled by the addition of apyrase, suggesting an adenosine diphosphate (ADP)-dependent mechanism. Treatment of platelets with TPO resulted in augmented binding of 125I-fibrinogen to intact platelets, with a 50% effect (EC50) occurring between 5 and 10 ng/mL. TPO-induced binding of fibrinogen to platelets was comparable in

degree with that observed by stimulation with 10 mumol/L ADP. In an immobilized collagen-platelet adhesion assay, a significant increase in the attachment of TPO-stimulated platelets was observed. This effect was dependent on the concentration of TPO. At 50 ng/mL of TPO, platelet attachment to collagen increased threefold compared with the buffer control. Furthermore, the presence of fibrinogen did not significantly alter TPO augmentation of the platelet-collagen interaction. This interaction was mediated by the Arg-Gly-Asp (RGD) adhesion recognition sequence, as it was completely abolished by 100 mumol/L of the RGDS peptide. A fraction of the TPO-dependent platelet attachment to a collagen-coated surface was insensitive to treatment with prostaglandin E1. Furthermore, %antibody% to alpha IIb integrin partially inhibited platelet attachment to collagen, suggesting that the integrin alpha IIb beta 3 participates in this association. These data indicate that TPO might function not only as a cytokine in megakaryocyte growth and differentiation, but may also participate in direct platelet activation and modulate platelet-extracellular matrix interactions.

7/3,AB/41 (Item 41 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08536402 96155135
Effect of conjugation methodology, carrier protein, and adjuvants on the immune response to Staphylococcus aureus capsular polysaccharides. Fattom A; Li X; Cho YH; Burns A; Hawwari A; Shepherd SE; Coughlin R; Winston S; Naso R
W.W. Karakawa Microbial Pathogenesis Laboratory, Univax Biologics Inc., Rockville, MD, USA.
Vaccine (ENGLAND) Oct %1995%, 13 (14) p1288-93, ISSN 0264-410X Journal Code: X6O
Contract/Grant No.: AI 33560-03, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Conjugate vaccines were prepared with S. aureus type 8 capsular polysaccharide (CP) using three carrier proteins: Pseudomonas aeruginosa exotoxin A (ETA), a non-toxic recombinant ETA (rEPA), and diphtheria toxoid (DTd). Adipic acid dihydrazide (ADH) or N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) was used as a spacer to link the CP to carrier protein. All conjugates gave a high immune response with a boost after the second immunization. Conjugates prepared with ADH gave higher %antibody% titers than conjugates prepared with SPDP. IgG1 was the primary subclass elicited by all conjugates regardless of the carrier protein or the conjugation method used to prepare the vaccines. The non-immunogenic CP and the conjugates were formulated with either monophosphoryl lipid A (%MPL%), QS21, or in Novasomes and evaluated in mice. While the adjuvants failed to improve the immunogenicity of the nonconjugated CP, a more than fivefold increase in the %antibody% levels was observed when these adjuvants were used with the conjugates. Significant rises in IgG2b and IgG3 were observed with all formulations. The enhancement of the immunogenicity and the IgG subclass shift, as seen with some adjuvants, may prove to be important in immunocompromised patients.

7/3,AB/42 (Item 42 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08533224 96164310
Establishment and characterization of a new human mesothelioma cell line (T-85) from malignant peritoneal mesothelioma with remarkable thrombocytosis [see comments]
Tange T; Hasegawa Y; Oka T; Sunaga S; Higashihara M; Matsuo K; Miyazaki H ; Shimosaka A; Okano A; Todokoro K
Department of Pathology, Faculty of Medicine, University of Tokyo, Japan. Pathol Int (AUSTRALIA) Nov %1995%, 45 (11) p791-800, ISSN 1320-5463 Journal Code: BXQ
Comment in Pathol Int 1996 Jun;46(6):471
Languages: ENGLISH
Document type: JOURNAL ARTICLE
A mesothelioma cell line, termed T-85, was established from a patient with malignant peritoneal mesothelioma and remarkable thrombocytosis (1.4 x 10(6)/mm3). Electron microscopically, two types of mesothelioma cells have been characterized; the major type of cells with dense-cored granules in the cytoplasm and the minor one with evenly dense granules. Immunologically, the cells showed staining for interleukin-6 (IL-6), cytokeratin, collagen type IV, vimentin, laminin, fibronectin and Factor VIII-related antigen. Quantitation by ELISA revealed a high concentration of IL-6 in T-85 cell culture supernatants. RT-polymerase chain reaction of T-85 cells showed two positive bands of cDNA at 628 and 251 base pairs indicating the constitutive expression of IL-6 and IL-6 receptor mRNA. Moreover, prominent pro-platelet process formation activity in T-85 cell culture supernatants indicated the presence of a thrombopoietic activity due mainly to IL-6 but not the c-%Mpl% ligand or erythropoietin. However, the fact that 15% of PPF activity remained in the supernatants treated with anti-IL-6 %antibody% indicated the presence of another thrombopoietic substance. T-85 is so far the first mesothelioma cell line derived from a case with remarkable thrombocytosis.

7/3,AB/43 (Item 43 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08509871 96138207
Inhibition of mouse placental lactogen-II release from placental cells by interleukin-1 after mid-pregnancy.
Yamaguchi M; Sakata M; Ogura K; Adachi K; Mammoto A; Miyake A Department of Obstetrics and Gynecology, Osaka University Medical School, Japan.
J Endocrinol (ENGLAND) Dec %1995%, 147 (3) p423-9, ISSN 0022-0795 Journal Code: I1J
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The effects of interleukin (IL)-1 and granulocyte-macrophage colony stimulating factor (GM-CSF), which are present in the mouse placenta, on the secretion of mouse placental lactogen (%mPL%)-1 and %mPL%-II by placental cells were tested in vitro. IL-1 alpha and IL-1 beta, 2.5 nmol/l each, significantly inhibited %mPL%-II secretion by cells from days 9 and 12 of pregnancy, but did not affect %mPL%-II secretion by cells from day 7 of pregnancy or %mPL% -I secretion by cells from days 7, 9 or 12 of pregnancy. GM-CSF had no effect on %mPL%-I and %mPL%-II secretion by cells from days 7, 9 or 12 of pregnancy. The inhibitory effects of IL-1 alpha and IL-1 beta on %mPL%-II secretion were completely eliminated by the addition of %antibodies% to IL-1 alpha and IL-1 beta respectively. Western blot analysis for %mPL%-II indicated that IL-1 alpha significantly reduced the intensity of the %mPL%-II band. Steady-state levels of %mPL%-II mRNA, assessed by Northern blot analysis, were reduced by incubation of placental cells from day 12 of pregnancy with 2.5 nmol/l IL-1 alpha for 5 days. Co-incubation of 0.25 pmol/l IL-1 alpha, 25 pmol/l IL-6, and 25 pmol/l tumor necrosis factor-alpha, each of which did not significantly inhibit %mPL%-II secretion by itself, together inhibited %mPL%-II secretion. These results suggest that IL-1, but not GM-CSF, is a potent inhibitor of %mPL% -II secretion after mid-pregnancy, and that the combined action of cytokines can inhibit %mPL%-II secretion.

7/3,AB/44 (Item 44 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

08481791 96115011
Posttranscriptional inhibition of mouse placental lactogen-II secretion by transforming growth factor beta 1: synergistic effects with epidermal growth factor and interleukin-6.
Yamaguchi M; Maeda T; Yoneda M; Nishizaki T; Miyake A Department of Obstetrics and Gynecology, Osaka University Medical School, Japan.
Biol Reprod (UNITED STATES) Aug %1995%, 53 (2) p373-9, ISSN 0006-3363 Journal Code: A3W
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We studied the effect of transforming growth factor beta 1 (TGF beta 1) on mouse placental lactogen (%mPL%)-I and %mPL%-II secretion by primary cultures of placental cells from Days 7, 9, and 12 of pregnancy. We also studied the effects of co-incubation of epidermal growth factor (EGF) or interleukin-6 (IL-6) with TGF beta 1 on %mPL%-I and %mPL%-II secretion. TGF beta 1 at 10 ng/ml did not affect %mPL%-I secretion by cells from Days 7 or 9 of pregnancy or %mPL%-II secretion by cells from Day 7 of pregnancy but significantly inhibited %mPL% -II secretion by cells from Days 9 or 12 of pregnancy. The lowest concentration of TGF beta 1 that significantly inhibited %mPL%-II secretion by cells from Days 9 or 12 of pregnancy was 1 ng/ml. Immunocytochemistry for %mPL% -II indicated that treatment of placental cells from Day 12 of pregnancy with 10 ng/ml TGF beta 1 significantly reduced the number of %mPL%-II-containing cells. Inhibition of %mPL% -II secretion by TGF beta 1 was eliminated completely by addition of an anti-TGF beta 1 %antibody%. Northern analysis showed that steady state levels of %mPL%-II mRNA were not reduced by incubation of placental cells from Day 12 of pregnancy with 10 ng/ml TGF beta 1 for 5 days. EGF at 10 ng/ml significantly inhibited %mPL%-II secretion by cells from Day 7 of pregnancy, and addition of 10 ng/ml TGF beta 1, which did not itself inhibit %mPL% -II secretion by those cells, enhanced the inhibition by EGF of %mPL%-II secretion.(ABSTRACT TRUNCATED AT 250 WORDS)

7/3,AB/45 (Item 45 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08417636 95218169
Thrombopoietin (c-%mpl% ligand) acts synergistically with erythropoietin, stem cell factor, and interleukin-11 to enhance murine megakaryocyte colony growth and increases megakaryocyte ploidy in vitro.
Broudy VC; Lin NL; Kaushansky K
University of Washington, Division of Hematology, Seattle, 98195, USA. Blood (UNITED STATES) Apr 1 %1995%, 85 (7) p1719-26, ISSN 0006-4971 Journal Code: A8G
Contract/Grant No.: R01 DK44194, DK, NIDDK; R01 DK43719, DK, NIDDK; R01 CA31615, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Thrombopoietin (Tpo), the ligand for the c-%mpl% receptor, is a major regulator of platelet production in

vivo. Treatment of mice with purified recombinant Tpo increases platelet count fourfold and expands colony-forming unit-megakaryocyte (CFU-Meg) numbers. Other cytokines including interleukin-3 (IL-3), IL-6, IL-11, erythropoietin (Epo), and stem cell factor (SCF) can stimulate megakaryopoiesis. Therefore, we examined the effects of recombinant murine Tpo in combination with these cytokines on megakaryopoiesis in vitro. Murine marrow cells were cultured in agar in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% horse serum and beta-mercaptoethanol in the presence of recombinant growth factors, and CFU-Meg colonies were counted on day 5. Megakaryocyte ploidy was analyzed using murine marrow cells cultured for 5 days in IMDM supplemented with 1% nutridoma-SP and recombinant growth factors. Megakaryocytes were identified by labeling with the 4A5 %antibody% and ploidy was analyzed by flow cytometry. Tpo supported the growth of CFU-Meg in a dose-dependent manner. Although the addition of SCF (50 ng/mL), Epo (2 U/mL), or IL-11 (50 ng/mL) alone exerted only a modest effect on CFU-Meg growth, the combination of SCF plus Tpo, Epo plus Tpo, or IL-11 plus Tpo resulted in a synergistic enhancement of the number of CFU-Meg colonies. IL-3 alone supported CFU-Meg colony growth, and the effects of IL-3 plus Tpo or IL-6 plus Tpo on colony growth appeared to be approximately additive. Fifty percent of megakaryocytes generated in cultures containing IL-3 or Epo displayed < or = 16 N ploidy. In contrast, cultures containing Tpo uniquely generated large numbers (30% to 35% of the total) of megakaryocytes with > or = 64N ploidy. These results show that Tpo stimulates both proliferation of committed megakaryocytic progenitor cells and maturation of megakaryocytes, and that two multipotent cytokines, SCF and IL-11, as well as a late-acting erythroid cytokine, Epo, can synergize with Tpo to stimulate proliferation of CFU-Meg.

7/3,AB/46 (Item 46 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08417123 95197478
The c-%Mpl% ligand (thrombopoietin) stimulates tyrosine phosphorylation of Jak2, Shc, and c-%Mpl%.
Drachman JG; Griffin JD; Kaushansky K
Department of Medicine, University of Washington, School of Medicine, Seattle 98195.
J Biol Chem (UNITED STATES) Mar 10 %1995%, 270 (10) p4979-82, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: R01DK43719, DK, NIDDK; CA31615, CA, NCI Languages: ENGLISH
Document type: JOURNAL ARTICLE
c-%Mpl% is a member of the cytokine receptor superfamily, expressed primarily on hematopoietic cells. Recently, the c-%Mpl% ligand was cloned and found to have thrombopoietic activity. In this paper we report that ligand binding induced tyrosine phosphorylation in BaF3 cells engineered to express the murine %Mpl% receptor (BaF3/mMpl). Phosphorylation occurred within 1 min at cytokine concentrations sufficient for proliferation of receptor-bearing cells. Using specific %antibodies% for immunoprecipitation and Western blotting, several of these phosphorylated proteins were identified. Shc and Jak2, known cytokine signaling molecules, and the c- %Mpl% receptor were shown to be major substrates for tyrosine phosphorylation. In contrast, phospholipase C-gamma and phosphatidylinositol 3-kinase displayed little and no tyrosine phosphorylation, respectively, after thrombopoietin stimulation. Co-immunoprecipitation studies demonstrated that Jak2 became physically associated with c-%Mpl% relatively late in the observed time course (20-60 min), significantly later than tyrosine phosphorylation of Jak2 (1-5 min). These results suggest that c-%Mpl% induces signal transduction pathways similar to those of other known cytokines. Additionally, in light of its late physical association with c-%Mpl% following ligand binding, Jak2 may not be the initiating tyrosine kinase in the thrombopoietin-induced signaling cascade.

7/3,AB/47 (Item 47 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08415493 95111122
The %Mpl% receptor is expressed in the megakaryocytic lineage from late progenitors to platelets.
Debili N; Wendling F; Cosman D; Titeux M; Florindo C; Dusanter-Fourt I; Schooley K; Methia N; Charon M; Nador R; et al
INSERM U362, Institut Gustave Roussy, Villejuif, France. Blood (UNITED STATES) Jan 15 %1995%, 85 (2) p391-401, ISSN 0006-4971 Journal Code: ABG
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The %Mpl% receptor (%Mpl% -R) is a cytokine receptor belonging to the hematopoietin receptor superfamily for which a ligand has been recently characterized. To study the lineage distribution of %Mpl%-R in normal hematopoietic cells, we developed a monoclonal %antibody% (designated M1 MoAb) by immunizing mice with a soluble form of the human %Mpl%-R protein. With few exceptions, %Mpl% -R was detected by indirect immunofluorescent analysis on all human leukemic hematopoietic cell lines with pluripotential and megakaryocytic phenotypes, but not on other cell lines. By immunoprecipitation and immunoblotting,

M1 MoAb recognized a band at 82 to 84 kD corresponding to the expected size of the glycosylated receptor. Among normal hematopoietic cells, M1 MoAb strongly stained megakaryocytes (MK) and %Mpl%-R was detected on platelets by indirect immunofluorescence staining or immunoblotting. On purified CD34+ cells, less than 2% of the population was stained, but the labeling was weak and just above the threshold of detection. However, dual-labeling with the M1 and antiplatelet glycoprotein MoAbs showed that most %Mpl%-R+/CD34+ cells coexpressed CD41a, CD61, or CD42a, suggesting that cell surface appearance of %Mpl%-R and platelet glycoproteins could be coordinated. M1-positive and M1-negative subsets were sorted from purified CD34+ cell populations. Colony assays showed that the absolute number of hematopoietic progenitors was extremely low and no primitive progenitors were present in the CD34+/%Mpl%-R+ fraction. However, this cell fraction was significantly enriched in low proliferative colony-forming units-MK. When the CD34+/%Mpl%-R+ fraction was grown in liquid culture containing human aplastic serum and a combination of growth factors, mature MK were seen as early as day 4, whereas the predominant cell population was erythroblasts on day 8. Similar data were also obtained with the CD34+/%Mpl%-R- fraction with, however, a delay in the time of appearance of both MK and erythroblasts. In conclusion, %Mpl%-R is a cytokine receptor restricted to the MK cell lineage. Its expression is low on CD34+ cells and these cells mainly correspond to late MK progenitors and transitional cells. These data indicate that the action of the %Mpl%-R ligand might predominate during the late stages of human MK differentiation.

7/3,AB/48 (Item 48 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08403314 95391968
Basic fibroblast growth factor mediates its effects on committed myeloid progenitors by direct action and has no effect on hematopoietic stem cells. Berardi AC; Wang A; Abraham J; Scadden DT
Division of Hematology/Oncology, New England Deaconess Hospital, Harvard Medical School, Boston, MA 02215, USA.
Blood (UNITED STATES) Sep 15 %1995%, 86 (6) p2123-9, ISSN 0006-4971 Journal Code: A8G
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Basic fibroblast growth factor or fibroblast growth factor-2 (FGF) has been shown to affect myeloid cell proliferation and hypothesized to stimulate primitive hematopoietic cells. We sought to evaluate the effect of FGF on hematopoietic stem cells and to determine if FGF mediated its effects on progenitor cells directly or through the induction of other cytokines. To address the direct effects of FGF, we investigated whether FGF induced production of interleukin-1 beta (IL-1 beta), tumor necrosis factor alpha, IL-6, granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor by two types of accessory cells, bone marrow (BM) fibroblasts and macrophages. We further evaluated whether %antibodies% to FGF-induced cytokines affected colony formation. To determine if FGF was capable of stimulating multipotent progenitors, we assessed the output of different colony types after stimulation of BM mononuclear cells (BMMC) or CD34+ BMMC and compared the effects of FGF with the stem cell active cytokine, kit ligand (KL). In addition, a subset of CD34+ BMMC with characteristics of hematopoietic stem cells was isolated by functional selection and their response to FGF was evaluated using proliferation, colony-forming, and single-cell polymerase chain reaction (PCR) assays. We determined that FGF had a stimulatory effect on the production of a single cytokine, IL-6, but that the effects of FGF on colony formation were not attributable to that induction. FGF was more restricted in its in vitro effects on BM progenitors than KL was, having no effect on erythroid colony formation. FGF did not stimulate stem cells and FGF receptors were not detected on stem cells as evaluated by single-cell reverse transcription PCR. In contrast, FGF receptor gene expression was detected in myeloid progenitor populations. These data support a directly mediated effect for FGF that appears to be restricted to lineage-committed myeloid progenitor cells. FGF does not appear to modulate the human hematopoietic stem cell.

7/3,AB/49 (Item 49 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08384381 95366230
Effect of DETOX as an adjuvant for melanoma vaccine.
Schultz N; Oratz R; Chen D; Zeleniuch-Jacquotte A; Abeles G; Bystryn JC Ronald O. Perelman Department of Dermatology, New York University Medical Center, New York 10016, USA.
Vaccine (ENGLAND) Apr %1995%, 13 (5) p503-8, ISSN 0264-410X Journal Code: X6O
Contract/Grant No.: 1RO1CA58484, CA, NCI; 1RO1CA60783, CA, NCI; FD-R-000632, FD, FDA
Languages: ENGLISH
Document type: CLINICAL TRIAL; CONTROLLED CLINICAL TRIAL; JOURNAL ARTICLE The identification of effective adjuvants is critical for tumor vaccine development. Towards this end, we examined whether the immunogenicity of a melanoma vaccine could

be potentiated by DETOX, an adjuvant consisting of monophosphoryl lipid A (%MPL%) and purified mycobacterial cell-wall skeleton (CWS). Nineteen patients with resected stage III melanoma were immunized with a polyvalent melanoma antigen vaccine (40 micrograms) admixed with DETOX, q3 wks x 4. Seven patients received vaccine + low-dose DETOX (10 micrograms %MPL% + 100 micrograms CWS) and 12 received vaccine + high-dose DETOX (20 micrograms %MPL% + 200 micrograms CWS). A non-randomized control group of 35 patients was treated similarly with 40 micrograms vaccine + alum. One week after the fourth vaccine immunization, melanoma %antibodies% were increased over baseline in 7/7 (100%) patients treated with vaccine + low-dose DETOX, 8/12 (67%) patients treated with vaccine + high-dose DETOX, and in 4/19 (21%) of vaccine + alum patients. For the entire DETOX group, the %antibody% response rate was 15/19 (79%) compared 4/19 (21%) in the alum group ($p < 0.001$). In contrast, a strong delayed-type hypersensitivity (DTH) response (> or = 15 mm increase in DTH response over baseline) was induced in 50% of the entire DETOX group versus in 47% of the alum group. Median disease-free (DF) survival for the entire DETOX group was 17.8 months compared with 32.1 months in the alum group ($p < 0.05$). In conclusion, DETOX markedly potentiated %antibody% but had little effect on DTH responses to melanoma vaccine immunization. It did not appear to improve disease-free survival in comparison to alum in this non-randomized study.

7/3,AB/50 (Item 50 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08348353 95315961

Association of anticardiolipin %antibodies% and abnormal nailfold capillaroscopy in patients with systemic lupus erythematosus. Bongard O; Bounameaux H; Miescher PA; De Moerloose P

Department of Medicine, University Hospital of Geneva, Switzerland. Lupus (ENGLAND) Apr %1995%, 4 (2) p142-4, ISSN 0961-2033 Journal Code: BRN

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Anticardiolipin %antibodies% (aCL) are found in about 40-50% of patients suffering from systemic lupus erythematosus (SLE) and their presence carries an increased risk of thromboembolism. Since there is a high prevalence of nailfold capillary abnormalities in patients with SLE, we studied the relationship between aCL and skin microcirculatory changes or vascular symptoms in 51 consecutive patients with SLE (49 women, 2 men, 34.8 +/- 13.7 years). Twenty-two patients (43.1%) had positive aCL (IgG 22 (5-60) GPL; IgM 5 (3-16.5) %MPL%; median titre and range) and 12 (54.5%) of them had abnormal capilloscopic findings. By contrast, among the 29 patients without aCL, only six (20.7%) had an abnormal capillaroscopy ($P = 0.027$). There was no correlation between either aCL or capillaroscopy and Raynaud's phenomenon. These results show a relationship between aCL and nailfold capillary changes in patients with SLE, suggesting a direct damage of the vascular endothelium by aCL.

7/3,AB/51 (Item 51 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08328043 95287052

Safety, immunogenicity, and efficacy of a recombinantly produced Plasmodium falciparum circumsporozoite protein-hepatitis B surface antigen subunit vaccine.

Gordon DM; McGovern TW; Krzych U; Cohen JC; Schneider I; LaChance R; Heppner DG; Yuan G; Hollingdale M; Slaoui M; et al

Department of Immunology, Walter Reed Army Institute of Research, Washington, DC, USA.

J Infect Dis (UNITED STATES) Jun %1995%, 171 (6) p1576-85, ISSN 0022-1899 Journal Code: IH3

Languages: ENGLISH

Document type: CLINICAL TRIAL; CLINICAL TRIAL, PHASE I; CONTROLLED CLINICAL TRIAL; JOURNAL ARTICLE

Twenty malaria-naive volunteers received a recombinant Plasmodium falciparum malaria vaccine (RTS,S) containing 19 NANP repeats and the carboxy terminus (amino acids 210-398) of the circumsporozoite (CS) antigen coexpressed in yeast with hepatitis B surface antigen. Ten received vaccine adjuvanted with alum, and 10 received vaccine adjuvanted with alum plus 3-deacylated monophosphoryl lipid A (%MPL%). Both formulations were well tolerated and immunogenic. %MPL% enhanced CS %antibody% levels (measured by ELISA, immunofluorescence, and inhibition of sporozoite invasion assays). After sporozoite challenge, 6 of 6 in the alum group and 6 of 8 in the alum-%MPL% group developed patent malaria. Protected subjects had higher levels of CS %antibody% titers on day of challenge than did nonprotected subjects. After immunization, 1 protected subject had increased cytotoxic T lymphocyte activity against CS and recall of memory T cell responses to RTS,S and selected CS.

7/3,AB/52 (Item 52 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08283640 95211633
Studies of the specificity and cross-reactions of %antibodies% to lipid A found in juvenile arthritis.
Miller JJ; Olds LC
Lucile Packard Children's Hospital, Stanford University School of Medicine, California 94305.
Clin Diagn Lab Immunol (UNITED STATES) Mar %1995%, 2 (2) p186-91, ISSN 1071-412X Journal Code: CB7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
This work was started to determine whether the immunoglobulin G (IgG) reactions with monophosphoryl lipid A (%MPL%) found in children with arthritis were due to contaminants, a specific site on lipid A, or polyspecific binding. Different lots of %MPL% were examined by electrophoresis and immunoblot. Competitive inhibition of enzyme-linked immunosorbent assays (ELISAs) by analogs of %MPL% and biologic materials of clinical interest was used to determine the specificity of the binding site and potential cross-reactions. IgG in all patient sera tested reacted with a single band just < 6.5 kDa on immunoblots of all lots of %MPL% tested. The ELISAs were inhibited best by analogs of lipid A with an exposed diglucosamine core and intact polar domains. The anti-%MPL% was also inhibited by fetal bovine collagen types I and II and in some instances by cardiolipin, but not by keratan sulfate, proteoglycan, or DnaK heat shock protein. Lot variation was a persistent technical problem, but no protein contaminant could be demonstrated in any lot. The ELISA and immunoblot results confirmed each other. Immunoblots detected a single band of %MPL% reactive with IgG. This %antibody% remains of interest because of its disease association and correlations and because it cross-reacts with collagen and cardiolipin.

7/3,AB/53 (Item 53 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08241083 94063958
Novel immunization protocol and ELISA screening methods used to obtain and characterize monoclonal %antibodies% specific for human light chain variable-region subgroups.
Abe M; Goto T; Wolfenbarger D; Weiss DT; Solomon A
Department of Medicine, University of Tennessee Medical Center/Graduate School of Medicine, Knoxville 37920-6999.
Hybridoma (UNITED STATES) Aug %1993%, 12 (4) p475-83, ISSN 0272-457X Journal Code: GFS
Contract/Grant No.: CA10056, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We have developed a novel immunization protocol for the production of a panel of high-affinity murine monoclonal %antibodies% (MoAbs) that are specific for each of the major human kappa and lambda light chain variable-region (VL) subgroups. Mice were injected with heat-precipitated human Bence Jones proteins or VL-related fragments emulsified in monophosphoryl lipid A (%MPL%) and trehalose dimycolate (TDM) at two- to four-week intervals over a seven-month period. A unique direct capturing enzyme-linked immunosorbent assay (ELISA) employing biotinylated monoclonal light chains was designed to select optimally immunized animals for hybridoma preparation and to screen culture supernatants for high-affinity anti-VL MoAbs. These methods have led to the generation of MoAbs that by ELISA react specifically with each of the four V kappa subgroups--V kappa I, V kappa II, V kappa III, and V kappa IV or five V lambda subgroups--V lambda I, V lambda II/V, V lambda III, V lambda IV, and V lambda VI. These reagents have been used successfully to establish, on the basis of VL subgroup, the monoclonal nature of serum or urinary immunoglobulins as well as those found in the cytoplasm or on the cell surface of monoclonal plasma cell or B-lymphocyte populations, respectively. The availability of anti-VL subgroup-specific MoAbs will facilitate the immunodiagnosis and study of patients with multiple myeloma, AL amyloidosis, and related B-cell proliferative disorders.

7/3,AB/54 (Item 54 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

08186809 94239432
Interaction of hamster submaxillary sialyl-Tn and Tn glycoproteins with Gal, GalNAc and GlcNAc specific lectins.
Wu AM; Shen F; Herp A; Wu JH
Glyco-Immunochemistry Research Lab., Chang-Gung Medical College, Tao-yuan, Taiwan.
Mol Immunol (ENGLAND) Apr %1994%, 31 (6) p485-90, ISSN 0161-5890 Journal Code: NG1
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Hamster submaxillary glycoprotein (HSM), one of the simplest glycoproteins among mammalian salivary mucins, is composed of approximately equivalent amounts of protein, hexosamine and sialic acid. The Thr and Ser residues in the protein core account for more than half of all of the amino acid residues, while Lys, Glu, Pro and Ala are the major components of the remaining portion of amino acids. The carbohydrate side chains of this mucous glycoprotein have mainly the NeuAc-GalNAc-(sialyl-Tn) sequence (HSM), and those

of the desialylated product (HSM-Tn) are almost exclusively unsubstituted GalNAc residues (Tn determinants). The binding properties of sialyl-Tn (HSM) and asialo-HSM (HSM-Tn) glycoproteins were tested by precipitin assay with Gal, GalNAc and GlcNAc specific lectins. The HSM-Tn completely precipitated Vicia villosa (VVL both B4 and mixture of A and B), Maclura pomifera (%MPL%), and Artocarpus integrifolia (Jacalin) lectins; less than 2 micrograms of HSM-Tn were required for precipitating 50% of 5.0-6.3 micrograms lectin nitrogen added. HSM-Tn also reacted well with Helix pomatia lectin (HPL), Wistaria floribunda lectin (WFL) and Abrus precatorius agglutinin (APA) and precipitated in each case over 81% of the lectin nitrogen added. The reactivity of HSM-Tn with other lectins (Ricinus communis, RCA1; Dolichol biflorus, DBL; Viscum album, ML-I; Arachis hypogaea, PNA, and Triticum vulgaris, WGA) was weak or negligible. The activity of sialyl-Tn (HSM) was more restricted; HSM reacted well with Jacalin, moderately with %MPL% and VVL-B4, but was inactive or only weakly with the other lectins used. These findings indicate that HSM and its desialylated product (HSM-Tn) are highly useful reagents for the differentiation of Tn and T/Gal specific lectins and for anti-T, Tn and Af monoclonal %antibodies%.

7/3,AB/55 (Item 55 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08162126 94186749
Expression of growth hormone-binding protein with a hydrophilic carboxyl terminus by the mouse placenta: studies in vivo and in vitro. Barnard R; Thordarson G; Lopez MF; Yamaguchi M; Edens A; Cramer SD; Ogren L; Talamantes F
Department of Biology, Sinsheimer Laboratories, University of California, Santa Cruz 95064.
J Endocrinol (ENGLAND) Jan %1994%, 140 (1) p125-35, ISSN 0022-0795 Journal Code: I1J
Contract/Grant No.: DK42361, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
GH-binding protein (GHBP) or GH receptor is present in numerous extrahepatic tissues in the rodent. From mid- to late gestation in the mouse, the maternal serum concentration of GHBP increases 30- to 50-fold. We have investigated whether the placenta might synthesize GHBP and potentially contribute to this increase. RNA was isolated from placentas and subjected to Northern analysis using a cDNA probe to the shared region of GHBP and GH receptor-encoding mRNAs. From day 8 to day 18 of gestation, the GHBP-encoding mRNA (1.4 kb) increased 45-fold in quantity. The GH receptor-encoding mRNA (4.2 kb) increased sixfold by day 14 and then remained steady until day 18. These changes which were not co-ordinated parallel reported changes in the steady-state concentrations of 1.4 and 4.2 kb mRNAs in maternal liver, suggesting shared regulatory factors. Extracts of freshly isolated trophoblasts were assayed for GHBP with a radioimmunoassay specific for GHBP with a hydrophilic carboxyl terminus. The cytosolic content of immunoreactive GHBP increased fourfold from mid- to late gestation. Trophoblasts were isolated from placentas and cultured for 2 days on collagen gels in defined medium. Cultured cells were at least 90% viable and secreted mouse placental lactogen-II (%mPL% -II). Immunocytochemistry was carried out simultaneously on cells cultured from day 7 to day 17 of gestation using a monoclonal %antibody% (MAb 4.3), which recognizes the hydrophilic C-terminus of GHBP. Cell-localized GHBP was present in trophoblasts cultured for 2 days, but GHBP was not detectable by radioimmunoassay or by immunoprecipitation in concentrated culture media from cultures treated with 100 ng mouse GH/ml or 100 ng %mPL%-II/ml or from untreated cultures. RNA was isolated from cells cultured in an identical manner to those analysed by immunocytochemistry. Three GH receptor/GHBP mRNA species of 8, 4.2 and 1.4 kb were observed. The quantity of 4.2 and 1.4 kb mRNAs did not change significantly in cultures from day 7 to day 15 of gestation but, in cultures from day 17 of gestation, the amount of 1.4 kb mRNA dropped significantly, while that of the 4.2 kb mRNA remained unchanged. GHBP- and GH receptor-encoding mRNAs are not co-ordinately regulated in vivo or in vitro. Although %mPL%-II was secreted into the medium by cultured trophoblasts, secretion of GHBP could not be detected. The culture medium may not contain the specific factors required for secretion of placental GHBP, or placental GHBP may not be destined for secretion.(ABSTRACT TRUNCATED AT 400 WORDS)

7/3,AB/56 (Item 56 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08132801 95195030
Co-localization of placental lactogen-I, placental lactogen-II, and proliferin in the mouse placenta at midpregnancy.
Yamaguchi M; Ogren L; Endo H; Soares MJ; Talamantes F
Department of Biology, University of California, Santa Cruz 95064. Biol Reprod (UNITED STATES) Dec %1994%, 51 (6) p1188-92, ISSN 0006-3363 Journal Code: A3W
Contract/Grant No.: HD-14966, HD, NICHD;

GM-08132, GM, NIGMS; HD-20676, HD, NICHD
Languages: ENGLISH
Document type: JOURNAL ARTICLE
This study was undertaken to determine whether mouse placental lactogen (%mPL%)-I, %mPL% -II, and proliferin (PLF) are expressed by the same population of placental giant cells at midpregnancy. Tissue sections from Day 9 of pregnancy were analyzed by double immunofluorescence staining. Sections were stained for PLF by use of a rhodamine-conjugated second %antibody%, and for %mPL%-I or %mPL%-II by use of a fluorescein-conjugated second %antibody% . All three proteins were present in most of the same giant cells. The distribution of %mPL%-I and PLF among giant cells in vitro was also examined. When placental cells from Day 7 of pregnancy were cultured for 5 days, > 90% of the cells that immunostained for %mPL%-I also immunostained for PLF on the first 3 days of culture. Thereafter, the percentage of cells that contained both proteins declined rapidly while the percentage that contained only PLF increased, suggesting continued differentiation of the cells in vitro. These data demonstrate that the same trophoblast giant cells express %mPL%-I, %mPL%-II, and PLF simultaneously at midpregnancy, suggesting that their gestational profiles in maternal blood during this period result at least partly from changes in gene expression in one population of cells and not from differentiation of several subsets of giant cells, each expressing only one member of the gene family.

7/3,AB/57 (Item 57 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08132570 95194523
Attachment of monophosphoryl lipid A (%MPL%) to cells and liposomes augments %antibody% response to membrane-bound gangliosides. Ravindranath MH; Morton DL; Irie RF
Laboratory of Glycolipid Immunotherapy, John Wayne Cancer Institute, Santa Monica, CA 90404.
J Autoimmun (ENGLAND) Dec %1994%, 7 (6) p803-16, ISSN 0896-8411 Journal Code: ADL
Languages: ENGLISH
Document type: JOURNAL ARTICLE
%Antibodies% to gangliosides are found in low levels in normal individuals, and attempts to augment their production have had limited success. Murine studies suggest that the %antibody% response to membrane-bound cryptic antigens, such as phospholipids and gangliosides, can be induced and augmented by attaching lipid A to membranes. Therefore, we assessed the ability of monophosphoryl lipid A, a non-toxic derivative of lipid A, to augment %antibody% response against membrane-associated gangliosides. Anti-ganglioside %antibodies% were IgM after the first and second immunizations; in contrast, anti-phospholipid %antibodies% were IgM after the first immunization and IgG after the second immunization. Mice (BALB/c) immunized with %MPL% -attached human cells as well as mice (C57BL/6J) immunized with %MPL% -attached syngeneic tumor cells (B16 melanoma) produced a significant IgM response. Mice (C57BL/6J) immunized with %MPL%-attached liposomes containing GM3 developed significantly higher IgM responses than those immunized with purified gangliosides, %MPL% or %MPL%-free B16 cells. However, the %antibody% response after immunization with %MPL%-GM3-liposomes is similar to that after immunization with %MPL% -attached tumor cells, even though the %MPL%-liposomes contained a 27-fold higher level of gangliosides than the tumor cells. Our results emphasize that co-expression of %MPL% with membrane-bound gangliosides is necessary to augment the anti-ganglioside %antibody% response. These findings may shed light on the elevated titers of anti-ganglioside IgM %antibodies% found in patients with motor neuron diseases, various neuropathies and classical ALS, and are relevant to clearance of circulating immunosuppressive gangliosides in cancer patients.

7/3,AB/58 (Item 58 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08119394 95165021
Anticardiolipin %antibodies% in leprosy.
Thawani G; Bhatia VN; Mukherjee A
Indian J Lepr (INDIA) Jul-Sep %1994%, 66 (3) p307-14, ISSN 0254-9395 Journal Code: IJL
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Eighty-four leprosy patients were studied clinically and for IgG and IgM anticardiolipin (aCL) %antibodies% . Following WHO criteria, 41 patients could be classified as multibacillary (MB) and 43 as paucibacillary (PB). Baseline levels of IgG and IgM %antibodies% were 27 +/- 4.8 GPL and 20 +/- 3.4 %MPL% per ml respectively. Comparing with these, 60.9% of MB and 39.5% of PB cases showed rise in IgG and IgM anticardiolipin %antibodies%; 19.5% of MB and 4.6% of PB sera showed rise in only IgG %antibodies%, while 4.8% of MB and 13.9% of PB cases showed rise only in IgM %antibodies%. Rise in aCL %antibodies% had no correlation with cardiovascular involvement, bacteriological index, reactive state and duration or regularity of treatment.

7/3,AB/59 (Item 59 from file: 155)

DIALOG(R)File 155:MEDLINE(R)
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08076047 95091063
Induction of humoral and cellular immunity to simian immunodeficiency virus: what are the requirements for protection?
Vaslin B; Le Grand R; Vogt G; Benveniste O; Gras G; Raques P; Stoeckel P; Salk PL; Salk J; Dormont D
Laboratoire de Neuropathologie Experimentale et Neurovirologie, Centre de Recherches du Service de Sante des Armees/Commissariat a l'Energie Atomique, Fontenay aux Roses, France.
Vaccine (ENGLAND) Sep %1994%, 12 (12) p1132-40, ISSN 0264-410X Journal Code: X6O
Languages: ENGLISH
Document type: JOURNAL ARTICLE
In an effort to produce a strong humoral and cellular immune response that might protect against simian immunodeficiency virus (SIV) infection, groups of five rhesus macaques each were immunized intramuscularly at 0, 2 and 6 months with 100 micrograms of an inactivated preparation of SIV/Delta B670 in either an oil-in-water emulsion with Ribi Detox, containing mycobacterial cell wall skeleton and monophosphoryl lipid A (CWS/%MPL%) (group A) or a water-in-oil emulsion with incomplete Freund's adjuvant, containing CWS/%MPL% for the first two injections (group B). Animals were challenged with 10-100 monkey ID50 of monkey-cell-grown SIVmac251 3 months after the last injection, along with a group of four unvaccinated controls. Group B animals demonstrated the strongest immune responses following immunization, including neutralizing %antibody% titres against the challenge virus ranging from 160 to 320 and SIV-specific ELISA titres ranging from 10(5)-10(6) on the day of challenge, as well as strong in vitro lymphoproliferative and interleukin-2 (IL-2) production responses to the immunogen. Neutralizing %antibody% was not detectable in group A animals, ELISA titres were lower (10(2)-10(4)), no in vitro lymphoproliferative responses were observed, and in vitro IL-2 production was less pronounced. No protection against challenge was observed in either group. Moreover, group B animals exhibited a more pronounced clinical response following challenge than either group A animals or controls, consisting of hyperthermia and a greater degree of lymphadenopathy on day 7, followed by hypothermia and generally higher levels of serum viraemia on day 14.(ABSTRACT TRUNCATED AT 250 WORDS)

7/3,AB/60 (Item 60 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07955508 94307348
Efficacy of tumor cell vaccine after incorporating monophosphoryl lipid A (%MPL%) in tumor cell membranes containing tumor-associated ganglioside. Ravindranath MH; Brazeau SM; Morton DL
Laboratory of Glycolipid Immunotherapy, John Wayne Cancer Institute, Santa Monica, California 90404.
Experientia (SWITZERLAND) Jul 15 %1994%, 50 (7) p648-53, ISSN 0014-4754 Journal Code: EQZ
Contract/Grant No.: PO1 CA12582, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Murine B16 melanoma expresses the ganglioside GM3. GM3 shed from tumor cells is immunosuppressive and promotes tumor growth. Reduction or elimination of the shed GM3 could be therapeutic, and the anti-GM3 %antibodies% may reduce and clear the shed ganglioside. To test this hypothesis, mice were challenged with tumor cells, with or without inducing anti-GM3 %antibody% response. Since gangliosides are poor immunogens and T-cell independent antigens, an adjuvant (monophosphoryl lipid A (%MPL%), a non-toxic lipid A of Salmonella), directed against B-cells, was employed. %MPL% was incorporated onto liposomes and into the surface membrane of B16 mouse melanoma cells; both are rich in GM3. C57BL/6J mice immunized with %MPL%-liposomes or %MPL%-B16 cells responded with elevated levels of anti-GM3 IgM. Non-immunized mice or mice immunized with B16 cells alone or ganglioside GM3 alone (without %MPL%) elicited poor anti-GM3 IgM response, confirming the GM3's immunologic crypticity and %MPL%'s immunopotentiating effect. %MPL%'s immunopotentiating effect was improved by coupling it to melanoma cell membranes. C57BL/6J mice were immunized with irradiated B16 alone or %MPL% alone or %MPL%-conjugated irradiated B16. After three weekly immunizations, each mouse received a challenge dose of viable syngeneic B16. Neither %MPL% alone nor B16 alone had a significant effect on tumor growth or host survival; however, administration of %MPL%-conjugated B16 cells significantly prevented tumor growth and prolonged survival. Our results indicate that %MPL%-incorporated B16 cells augment the anti-GM3 IgM response, which may reverse GM3-induced immunosuppression by eliminating tumor-derived GM3, and restore immunocompetence.

7/3,AB/61 (Item 61 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07902658 94226121
Anticardiolipin %antibody%-associated cerebral infarction in cirrhosis: clearance of anticardiolipin %antibody% after liver transplantation. Talenti DA; Falk GW; Carey WD; Marchant K

Department of Gastroenterology, Cleveland Clinic Foundation, Ohio. Am J Gastroenterol (UNITED STATES) May %1994%, 89 (5) p785-8, ISSN 0002-9270 Journal Code: 3HE
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We report a 47-yr-old woman with a 30-yr history of cryptogenic cirrhosis who sustained a cerebral infarction shortly before undergoing liver transplantation. Anticardiolipin %antibody% titers were positive prior to transplantation (IgG = 24 GPL; IgM > 80 %MPL%; IgA < 10 APL). After liver transplantation, however, the titers dropped to normal (< 10 PL units). This report suggests that cirrhotic patients with high anticardiolipin %antibody% titers may be at risk for thrombotic events, and that this tendency may be reversed by liver transplantation or post-transplant immunosuppressive agents.

7/3,AB/62 (Item 62 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07587665 93322093
Lipopolysaccharide, lipid A, and liposomes containing lipid A as immunologic adjuvants.
Alving CR
Department of Membrane Biochemistry, Walter Reed Army Institute of Research, Washington, DC.
Immunobiology (GERMANY) Apr %1993%, 187 (3-5) p430-46, ISSN 0171-2985 Journal Code: GH3
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL Numerous studies have demonstrated that most or all of the potent adjuvant activity of Gram-negative bacterial endotoxin resides in the lipid A moiety of lipopolysaccharide (LPS). Synthetic analogues of lipid A have provided insights into structure-activity relationships. Several cellular mechanisms of LPS and lipid A adjuvant activities have been identified. Activation of macrophages by LPS or lipid A results in cytokine secretions that enhance the immune response. LPS and lipid A cause recruitment of antigen-presenting cells, particularly macrophages. Liposomes containing lipid A serve as an in vivo adjuvant to recruit increased numbers of macrophages. Liposomal lipid A that has been phagocytized by cultured macrophages also serves as an "intracellular adjuvant" to cause increased immunologic presentation of liposomal antigen by the macrophages to specific T lymphocytes. Lipid A can abolish suppressor T cell activity, resulting in increased immune responses to polysaccharide antigens. Upon combination of lipid A or lipid A analogues with nonionic block polymers, modulation of murine %antibody% isotypes can be achieved with %antibodies% against a variety of antigens in vivo. Liposomes containing monophosphoryl lipid A (%MPL%) have been utilized in a phase I clinical trial of a proposed malaria vaccine in humans. The liposomal malaria vaccine resulted in very high levels of %antibodies% against the malarial antigen, and despite the presence of huge amounts of %MPL% (up to 2.2 mg), the liposomal lipid A was nonpyrogenic and safe for use in humans. Lipid A and lipid A analogues, and liposomes or other carriers containing lipid A, have shown considerable promise both as adjuvants for immunization of animals and for human vaccines.

7/3,AB/63 (Item 63 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07573063 93299998
[Pulmonary thromboembolism in antiphospholipid syndrome] Pulmonale Thromboembolien bei Antiphospholipid-Syndrom.
Dumoulin FL; Daniel WG; Lichtlen P; Schmidt RE
Abteilung Immunologie und Transfusionsmedizin, Zentrums Innere Medizin und Dermatologie der Medizinischen Hochschule Hannover.
Dtsch Med Wochenschr (GERMANY) Jun 18 %1993%, 118 (24) p903-6, ISSN 0012-0472 Journal Code: ECL
Languages: GERMAN Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE English Abstract
A 37-year-old woman in acute right heart failure had experienced systemic venous thromboses for 17 years, five miscarriages and repeated pulmonary emboli. For the last 7 years she had been treated symptomatically for pulmonary hypertension. The platelet count was 62,000/microliters, thromboplastin time under phenprocoumon was 22%, partial thromboplastin time was 72 s. Despite anticoagulation with phenprocoumon and heparin (7,500 IU two times daily subcutaneously) new pulmonary emboli occurred and platelet count fell to 12,000/microliters. An increased titre for anticardiolipin %antibodies% (IgG > 320 GPL U/l, IgM 8 %MPL% U/l), antinuclear (1:640) and anti-ds-DNA %antibodies% (> 200 IU/ml) with simultaneous complement consumption suggested secondary antiphospholipid syndrome associated with lupus erythematodes. Treatment with prednisolone (150 mg/d), immunoglobulins (20 mg/d intravenously for 5 days) and heparin (25,000 IU/24 h intravenously) achieved an increase in platelet count to 200,000/microliters within 10 days, but fell again when the prednisolone dose was reduced, recovering under azathioprine, 150 mg/d. Four weeks later the patient died of renewed acute right heart failure.

7/3,AB/64 (Item 64 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07451125 93117758

Longitudinal survey of anticardiolipin %antibodies% in systemic lupus erythematosus. Relationships with clinical manifestations and disease activity in an Italian series.

Picillo U; Migliaresi S; Marcialis MR; Longobardo A; La Palombara F; Tirri G

Institute of Clinical Medicine, 1st Faculty of Medicine, University of Naples, Italy.

Scand J Rheumatol (NORWAY) %1992%, 21 (6) p271-6, ISSN 0300-9742 Journal Code: UD1

Languages: ENGLISH

Document type: JOURNAL ARTICLE

One hundred and two patients suffering from systemic lupus erythematosus were observed over 5-140 months. IgG and IgM anticardiolipin %antibodies% (aCL) were searched for by ELISA in 448 serum samples, and were found in 88 (86.3%) patients on at least one occasion. Changes of aCL levels and isotypes were recorded in most patients. In patients with medium (20-80 U. GPL and/or %MPL%) and high (> 80 U. GPL and/or %MPL%) aCL levels associations with thrombosis, neurological manifestations, cerebrovascular disease, prolonged aPTT, and thrombocytopenia were found. A highly significant statistical association was found between IgG aCL and the occurrence of active disease ($p < 0.0001$).

7/3,AB/65 (Item 65 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07391976 91022550

Special report. The Second International Anti-cardiolipin Standardization Workshop/the Kingston Anti-Phospholipid %Antibody% Study (KAPS) group. Harris EN

Department of Medicine, University of Louisville, Kentucky 40292. Am J Clin Pathol (UNITED STATES) Oct %1990%, 94 (4) p476-84, ISSN 0002-9173 Journal Code: 3FK

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Forty-three laboratories in 13 countries participated in a workshop to determine the degree of agreement between laboratories performing anticardiolipin tests. Each laboratory received freeze-dried aliquots of three samples labeled G1 (107 GPL units), G2 (20 GPL units), and G3 (6 GPL units) to be used as reference standards in the IgG assay, and three samples labeled M1 (106 %MPL% units), M2 (21 %MPL% units), and M3 (5 %MPL% units) as references for the IgM assay. Participating laboratories were divided into 8 groups and serum samples were exchanged between laboratories in each group. For IgG anticardiolipin, results were reported as: high, IgG positive for samples with optical absorbance readings exceeding G1; medium, IgG positive for samples with readings between G1 and G2; low, IgG positive between G2 and G3, and negative, if less than G3. In like manner, samples were defined as high-, medium-, or low-IgM positive, with reference to standards M1, M2, and M3. An index of agreement was computed to determine the degree of agreement between laboratories in each group. Interlaboratory agreement was excellent in each category assessed. For high positive and negative IgG and IgM results, the index of agreement exceeded 90%, and for medium and low positive results, agreement exceeded 75%. The overall index of agreement between laboratories exceeded 90%. The researchers conclude that the use of these six standards to obtain a semiquantitative measure of anticardiolipin positivity will enable good interlaboratory agreement in reporting anticardiolipin results.

7/3,AB/66 (Item 66 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07365337 92286029

Comparative analysis of the immunostimulatory properties of different adjuvants on the immunogenicity of a prototype parainfluenza virus type 3 subunit vaccine.

Ewasyshyn M; Caplan B; Bonneau AM; Scollard N; Graham S; Usman S; Klein M Connaught Centre for Biotechnology Research, Willowdale, Ontario, Canada. Vaccine (ENGLAND) %1992%, 10 (6) p412-20, ISSN 0264-410X Journal Code: X60

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The immunogenicity of a parainfluenza virus type 3 (PIV-3) subunit vaccine consisting of affinity-purified haemagglutinin-neuraminidase (HN) and fusion (F) surface glycoproteins was tested in guinea-pigs and hamsters. The ability of several different immunopotentiating agents to enhance the %antibody% response of animals to the PIV-3 surface glycoproteins was evaluated. The immunity induced by HN and F alone was compared with the response elicited by purified proteins combined with Freund's complete adjuvant, aluminium phosphate, Syntex's threonyl-muramyl dipeptide (MDP) SAF-MF formulation, or Ribi's adjuvant formulation containing BCG cell wall skeleton (CWS), trehalose dimycolate (TDM) and monophosphoryl lipid A (%MPL%) in a 2% squalene-in-water emulsion. Purified proteins were also incorporated into three different liposome formulations prepared by the detergent dialysis procedure. Immunization of guinea-pigs and hamsters with two

15 micrograms doses of the PIV-3 surface glycoproteins administered in the absence of adjuvant elicited high haemagglutination inhibition, neutralization and anti-fusion titres. The liposome preparations failed to enhance the %antibody% titres. Ribi's adjuvant formulation was effective at inducing a good secondary response to the purified proteins while the immunostimulatory effects of aluminium phosphate, Syntex and Freund's adjuvants were clearly demonstrated in both primary and secondary responses. When administered without adjuvant, a 15 microgram dose of the HN and F mixture was capable of protecting hamsters against live virus challenge. The immunoprotective dose of the purified proteins could be reduced to at least 0.1 microgram by the addition of aluminium phosphate, Syntex or Freund's adjuvants.

7/3,AB/67 (Item 67 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07343103 90322912
Molecular cloning and expression of rat placental lactogen-I complementary deoxyribonucleic acid.
Robertson MC; Croze F; Schroedter IC; Friesen HG
Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada.
Endocrinology (UNITED STATES) Aug %1990%, 127 (2) p702-10, ISSN 0013-7227 Journal Code: EGZ
Contract/Grant No.: HD-07843-14, HD, NICHD
Languages: ENGLISH
Document type: JOURNAL ARTICLE
A full-length cDNA clone for rat placental lactogen I (rPL-I) has been isolated from a phage lambda gt11 library containing cDNA synthesized from day 11 rat placental mRNA. By Northern blot analysis the rPL-I cDNA clone hybridizes to a 1.0-kilobase placental mRNA and appears as early as day 10 of gestation. Maximal expression of this mRNA was observed in day 11 and 12 placenta, and faint hybridization of the rPL-I cDNA was also detected in day 18 to term placenta. In contrast, the mouse clone hybridized to mRNA for mouse PL-I (%mPL% -I) only in day 10 mouse placenta (9). In vitro translation of rPL-I mRNA produced by transcription of the cDNA template yielded a 27-kDa polypeptide the size of the expected precursor protein which was immunoprecipitated by a monoclonal %antibody% to rPL-I. The rPL-I cDNA nucleotide sequence has been determined. The sequence is very similar to that for %mPL% -I and contains an open reading frame encoding a polypeptide of 230 amino acids compared to 224 for %mPL%-I. Comparison of the predicted primary translation product of rPL-I mRNA with that of %mPL% -I mRNA revealed that rPL-I shares 73% identity to %mPL%-I at the amino acid level. The predicted rPL-I protein shares 41% amino acid identity with rPL-II precursor, 24% with rat prolactin-like protein A, 26% with rat prolactin-like protein B, and 31% with rat PRL. In situ hybridization studies indicated that mRNA for rPL-I was present in a few rapidly dividing cells as early as day 8 of gestation, and by day 9 could be localized to giant cells which surround the conceptus.

7/3,AB/68 (Item 68 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

07331827 93018880
Adjuvant-dependent immune response to malarial transmission-blocking vaccine candidate antigens [published erratum appears in J Exp Med 1993 Feb 1;177(2):following 576]
Rawlings DJ; Kaslow DC
Molecular Vaccine Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892. J Exp Med (UNITED STATES) Nov 1 %1992%, 176 (5) p1483-7, ISSN 0022-1007 Journal Code: I2V
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Immune responses in major histocompatibility complex (MHC)-disparate congenic mouse strains immunized with sexual stage malaria parasites or purified recombinant protein were adjuvant dependent. Whereas mice exhibited a limited %antibody% response to immunization with newly emerged Plasmodium falciparum gametes in Freund's adjuvant, all five congenic mouse strains responded to several transmission-blocking vaccine candidate antigens, when parasites were emulsified in a monophosphoryl lipid A (%MPL%) and trehalose dimycolate (TDM) adjuvant. The humoral response in those animals immunized with the antigen in a %MPL%/TDM adjuvant was helper T cell dependent, as evident by boosting of the %antibody% response after a second immunization. If the immunogen consisted of purified recombinant protein, then the immune response was not MHC class II limited in mice immunized with either complete Freund's adjuvant or TDM/%MPL% . The potential role of adjuvants in overcoming apparent immune nonresponsiveness and the implications for development of a malaria transmission-blocking vaccine are discussed.

7/3,AB/69 (Item 69 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07236673 93124830
The selection of an adjuvant emulsion for

polyclonal %antibody% production using a low-molecular-weight antigen in rabbits. Smith DE; O'Brien ME; Palmer VJ; Sadowski JA
School of Nutrition, Tufts University, Boston, MA 02111.
Lab Anim Sci (UNITED STATES) Dec %1992%, 42 (6) p599-601, ISSN 0023-6764 Journal Code: KYS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Although Freund's adjuvant has been used for decades as an immune enhancer in rabbits, adverse physiologic side effects have prompted the search for more suitable alternatives. We used osteocalcin, a bovine bone protein (M.W. 5,800), as the test antigen to evaluate four adjuvant regimens: a) primary inoculation with complete Freund's adjuvant (CFA) followed by three boosts with incomplete Freund's adjuvant (IFA), b) four serial inoculations with RIBI %MPL% +TDM+CWS adjuvant, c) four serial inoculations with TiterMax #R-1, and d) primary inoculation (only) with TiterMax #R-1. The %antibody% yield associated with the CFA/IFA regimen (mean OD = 2.152) was at least sixfold that of either TiterMax (mean OD = 0.358) or RIBI (mean OD = 0.239) multiple injection regimens. No %antibody% response was observed after the single injection of TiterMax antigen emulsion. Maximal %antibody% production occurred rapidly in response to Freund's adjuvant (day 31) as compared with TiterMax (day 74) and RIBI (day 66).

7/3,AB/70 (Item 70 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07216298 93089631
Monophosphoryl lipid A-induced immune enhancement of Brucella abortus salt-extractable protein and lipopolysaccharide vaccines in BALB/c mice. Tabatabai LB; Pugh GW Jr; Stevens MG; Phillips M; McDonald TJ
USDA, Agricultural Research Service, National Animal Disease Center, Ames, IA 50010.
Am J Vet Res (UNITED STATES) Oct %1992%, 53 (10) p1900-7, ISSN 0002-9645 Journal Code: 40C
Languages: ENGLISH
Document type: JOURNAL ARTICLE
A study was conducted to determine the effect of monophosphoryl lipid A (%MPL%) and trehalose dimycolate (TDM) as adjuvants on the protective responses in BALB/c mice vaccinated with Brucella abortus salt-extractable protein (BCSP) or proteinase-K-treated B abortus lipopolysaccharide (PKLPS). Mice were vaccinated with different doses of BCSP or PKLPS given alone or in combination with %MPL% or TDM. Mice were challenge-exposed 4 weeks later with virulent B abortus strain 2308. Two weeks after challenge exposure, the number of B abortus colony-forming units (CFU) per spleen, spleen weights, and spleen cell interleukin 1 production were measured. Serum IgG and IgM concentrations specific for vaccinal immunogens were measured before and after challenge exposure with B abortus. Spleen weights and mean B abortus CFU per vaccine group were significantly lower in BCSP- and PKLPS-vaccinated mice, compared with those of nonvaccinated control mice. Monophosphoryl lipid A enhanced the suppression of splenic infection when given with the BCSP vaccine, but not when given with the PKLPS vaccine. Trehalose dimycolate had no effect on mean CFU when given with BCSP, but incorporation of TDM resulted in a significant increase in mean CFU when given with PKLPS. Spleen weights in BCSP- or PKLPS-vaccinated mice were not different when these vaccines were combined with %MPL% or TDM. Because of the wide variation in the results, we could not conclude that vaccination with BCSP or PKLPS alone, or in combination with %MPL% altered spleen cell interleukin-1 production in B abortus-infected mice.(ABSTRACT TRUNCATED AT 250 WORDS)

7/3,AB/71 (Item 71 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07170646 93020791
%Antibodies% to lipid A in pauciarticular juvenile arthritis: clinical studies.
Miller JJ 3d; Olds LC
Children's Hospital, Stanford University School of Medicine, CA. J Rheumatol (CANADA) Jun %1992%, 19 (6) p959-63, ISSN 0315-162X Journal Code: JWX
Contract/Grant No.: 2 P60 AM 20610, AM, NIADDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
IgG %antibodies% to monophosphoryl lipid A (%MPL%) are more concentrated in synovial fluids than in the blood of children with pauciarticular juvenile arthritis. Correlations between IgG anti-%MPL% levels and numbers of inflamed joints were highly significant in 2 of 10 patients followed for 8 years and suggestive in 2 patients followed for shorter periods. A fifth patient had a correlation between IgM anti-%MPL% and joint count.

7/3,AB/72 (Item 72 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07012823 92112336
Immunization against anthrax with Bacillus anthracis protective antigen combined with adjuvants.
Ivins BE; Welkos SL; Little SF; Crumrine MH; Nelson GO
Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick,

Frederick, Maryland 21702-5011. Infect Immun (UNITED STATES) Feb %1992%, 60 (2) p662-8, ISSN 0019-9567 Journal Code: GO7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The protective efficacy of immunization against anthrax with Bacillus anthracis protective antigen (PA) combined with different adjuvants was tested in Hartley guinea pigs and CBA/J and A/J mice. Adjuvant components derived from microbial products that were tested included threonyl-muramyl dipeptide (threonyl-MDP); monophosphoryl lipid A (%MPL%); trehalose dimycolate (TDM); and the delipidated, deproteinized, cell wall skeleton (CWS) from either Mycobacterium phlei or the BCG strain of Mycobacterium bovis. Non-microbially derived adjuvants tested included aluminum hydroxide and the lipid amine CP-20,961. In guinea pigs, all adjuvants and adjuvant mixtures enhanced %antibody% titers to PA as well as survival after a parenteral challenge of virulent B. anthracis Ames spores. In contrast, PA alone or combined with either aluminum hydroxide or CP-20,961 failed to protect mice. Vaccines containing PA combined with threonyl-MDP or %MPL% -TDM-CWS protected a majority of female CBA/J mice. Statistical analysis of survival data in the guinea pigs indicated that PA-%MPL%-CWS and PA-%MPL% -TDM-CWS were more efficacious than the currently licensed human anthrax vaccine.

7/3,AB/73 (Item 73 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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06964477 91203413
Use of adjuvant containing mycobacterial cell-wall skeleton, monophosphoryl lipid A, and squalane in malaria circumsporozoite protein vaccine.
Rickman LS; Gordon DM; Wistar R Jr; Krzych U; Gross M; Hollingdale MR; Egan JE; Chulay JD; Hoffman SL
Division of Infectious Diseases, National Naval Medical Center, Bethesda, Maryland 20852.
Lancet (ENGLAND) Apr 27 %1991%, 337 (8748) p998-1001, ISSN 0140-6736 Journal Code: LOS
Languages: ENGLISH
Document type: CLINICAL TRIAL; JOURNAL ARTICLE
Human immune responses to modern synthetic and recombinant peptide vaccines administered with the standard adjuvant, aluminum hydroxide, tend to be poor, hence the search for better adjuvants. %Antibody% responses to a Plasmodium falciparum circumsporozoite (CS) protein vaccine, R32NS1(81), administered with an adjuvant containing cell-wall skeleton of mycobacteria and monophosphoryl lipid A in squalane (%MPL%/CWS) have been compared to responses to the same immunogen administered with aluminum hydroxide. 2 weeks after the third dose the following indices were greater in the 5 patients who received %MPL%/CWS than in controls (p less than 0.05): the geometric mean concentration (2.0 vs 25.4 microgram/ml) and avidity index of %antibodies% to the P falciparum CS protein by ELISA, the geometric mean titre to P falciparum sporozoites by IFAT (1/115 vs 1/1600), and the geometric mean inhibition of sporozoite invasion of hepatoma cells in vitro (37.6 vs 90.3%). For R32NS1(81) %MPL%/CWS is superior to aluminum hydroxide as an adjuvant, and the data support the evaluation of this complex as an adjuvant for other vaccines.

7/3,AB/74 (Item 74 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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06939283 91194288
An evaluation of several adjuvant emulsion regimens for the production of polyclonal antisera in rabbits.
Johnston BA; Eisen H; Fry D
Fred Hutchinson Cancer Research Center, Seattle, WA.
Lab Anim Sci (UNITED STATES) Jan %1991%, 41 (1) p15-21, ISSN 0023-6764 Journal Code: KYS
Contract/Grant No.: S07-RR-05761, RR, NCRR; P01-CA-28151, CA, NCI; P30-CA-15704, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Emulsion adjuvants have been used for production of polyclonal antisera in rabbits (Oryctolagus cunniculi) for decades. Complete Freund's adjuvant has a reputation as a very effective immunoenhancer, but adverse physiological effects, including fever, inflammation and sterile abscess formation, have prompted a search for alternatives to complete Freund's. In this study, we quantitatively compared five adjuvant regimens: (a) a primary inoculation with complete Freund's followed by three boosts with incomplete Freund's; (b) four serial inoculations of incomplete Freund's adjuvant augmented with 6-bromoguanisine; (c) four serial inoculations with RIBI's %MPL% + TDM + CWS adjuvant emulsion; (d) four serial inoculations with Montanide ISA 50 emulsion; and (e) four serial inoculations with Montanide ISA 70 emulsion. We chose a small (12 amino acid) chain polypeptide coupled to bovine serum albumin as our test antigen. When compared, no system could be seen to be significantly better than a regimen of a primary immunization with complete Freund's adjuvant followed by serial reimmunization with incomplete Freund's adjuvant. The commercially available RIBI adjuvant produced significantly lower %antibody% levels, while other systems produced essentially equivalent levels. With all five adjuvants, %antibody% quantities plateaued after the second injection and further immunization did not increase titers significantly.

Boost injections did yield greater intradermal tissue reaction than primary inoculations, and intramuscular inoculum volumes of 0.4 cc caused chronic lesions still detectable by the gross necropsy 2 weeks after the final injection.

7/3,AB/75 (Item 75 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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06927142 92060231
Comparison of %antibody% response by use of synthetic adjuvant system and Freund complete adjuvant in rabbits.
Mallon FM; Graichen ME; Conway BR; Landi MS; Hughes HC
Department of Laboratory Animal Science, SmithKline & French Laboratories, King of Prussia, PA 19406.
Am J Vet Res (UNITED STATES) Sep %1991%, 52 (9) p1503-6, ISSN 0002-9645 Journal Code: 40C
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Two commercially available synthetic adjuvant systems, trehalose dimycolate (TDM) and TDM + monophosphoryl lipid A (%MPL%), were compared with Freund complete adjuvant (FCA) for the ability to stimulate %antibody% production in New Zealand White rabbits (Oryctolagus cuniculus). In addition, each animal was evaluated for adverse reactions. The antigen, rat liver microsomal epoxide hydrolase, was administered SC emulsified with FCA, TDM, or TDM + %MPL%. Serum %antibody% titers were stimulated with all 3 adjuvant-antigen combinations. The highest titer was produced by use of FCA; TDM + %MPL% produced an intermediate response, and TDM produced the lowest titer. All of the rabbits immunized with FCA developed sterile subcutaneous abscesses. Rabbits immunized with TDM or TDM + %MPL% developed no abscesses, and only slight reactions at the injection site. The synthetic adjuvant system TDM + %MPL% is recommended for use in rabbits, considering its adequate stimulation of %antibody% production with minimal adverse reactions.

7/3,AB/76 (Item 76 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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06920782 91244473
Differential effects of monophosphoryl lipid A on expression of suppressor T cell activity in lipopolysaccharide-responsive and lipopolysaccharide-defective strains of C3H mice.
Ekwunife FS; Taylor CE; Fauntleroy MB; Stashak PW; Baker PJ Department of Natural Sciences, University of Maryland-Eastern Shore, Princess Anne 21853.
Infect Immun (UNITED STATES) Jun %1991%, 59 (6) p2192-4, ISSN 0019-9567 Journal Code: GO7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Lipopolysaccharide (LPS)-responsive and LPS-defective strains of C3H mice did not differ in the capacity to make an %antibody% response to type III pneumococcal polysaccharide or in the degree of thymus-derived suppressor cell (Ts) activity generated following exposure to type III pneumococcal polysaccharide. However, treatment with monophosphoryl lipid A (%MPL%) abolished the expression of Ts function in LPS-responsive but not LPS-defective mice. Since this effect was elicited by different preparations of %MPL%, it appears to be a general property of %MPL% mediated by direct action of %MPL% on activated Ts.

7/3,AB/77 (Item 77 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

06868516 92118741
A comparison of the immunomodulating properties of two forms of monophosphoryl lipid A analogues.
Johnson AG; Tomai MA; Chen YF; Odean M
Department of Medical Microbiology/Immunology, University of Minnesota, Duluth 55812.
J Immunother (UNITED STATES) Dec %1991%, 10 (6) p398-404, ISSN 1053-8550 Journal Code: AZ0
Contract/Grant No.: AI 25810, AI, NIAID; AG 06198, AG, NIA Languages: ENGLISH
Document type: JOURNAL ARTICLE
This investigation compared the immunomodulating activities of two forms monophosphoryl lipid A, which are analogues of bacterial lipopolysaccharides with little or no toxicity. Tested were a synthetic compound designated 504 and a purified compound, isolated from bacterial cell walls designated %MPL% . Both of these clinical adjuvant candidates were effective in mice in exerting strong immunomodulating activity in the following areas: (a) enhancing %antibody% production in young and aging mice; (b) suppressing %antibody% formation under different experimental conditions; (c) activating macrophages to secrete interleukin 1, hydrogen peroxide, and superoxide anion; and (d) stimulating proliferation of spleen cells from C3H/HeN mice. Both exhibited considerably reduced toxicity in LD50 assays when compared to native lipopolysaccharides (LPS). The LD50 for %MPL% was 225 times and that of compound 504, 40 times that of native LPS in the exquisitely sensitive, galactosamine-loaded C57BL/6 murine strain.

7/3,AB/78 (Item 78 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06805498 92013000
Evaluation of monophosphoryl lipid A (%MPL%) as an adjuvant. Enhancement of the serum %antibody% response in mice to polysaccharide-protein conjugates by concurrent injection with %MPL%.
Schneerson R; Fattom A; Szu SC; Bryla D; Ulrich JT; Rudbach JA; Schiffman G; Robbins JB
Laboratory of Developmental and Molecular Immunity, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.
J Immunol (UNITED STATES) Oct 1 %1991%, 147 (7) p2136-40, ISSN 0022-1767 Journal Code: IFB
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Concurrent injection of monophosphoryl lipid A (%MPL%) in saline or as an oil-in-water emulsion enhanced both the primary and secondary serum %antibody% responses to the capsular polysaccharide (CP) components of seven conjugates: the enhanced responses were Ag-specific. In contrast, %MPL% did not enhance the serum %antibody% response to five of the six unconjugated CP. %MPL% and trehalose dimycolate injected concurrently with the unconjugated Vi CP of Salmonella typhi (Vi) enhanced the serum %antibody% response to that Ag. %MPL% further enhanced the Vi %antibody% levels when injected with conjugates of this CP. The serum %antibody% responses to Pseudomonas aeruginosa exotoxin A, used as the carrier protein for the Staphylococcus aureus types 5 and 8 conjugates, were also enhanced by %MPL%. %MPL% in oil-in-water emulsion was generally more effective than when administered in saline.

7/3,AB/79 (Item 79 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06762845 91332940
Effect of cell wall skeleton and monophosphoryl lipid A adjuvant on the immunogenicity of a murine B16 melanoma vaccine.
Johnston D; Bystryn JC
Department of Dermatology, New York University School of Medicine, NY 10016.
J Natl Cancer Inst (UNITED STATES) Sep 4 %1991%, 83 (17) p1240-5, ISSN 0027-8874 Journal Code: J9J
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We examined the effect of a new adjuvant consisting of purified mycobacterial cell wall skeleton (CWS) and monophosphoryl lipid A (%MPL%) on the immunogenicity of a murine melanoma vaccine. C57BL/6 mice were immunized to partially purified B16 melanoma vaccine given alone or together with different dose levels of adjuvant, or with saline or adjuvant alone. Humoral response, delayed-type hypersensitivity (DTH), in vitro cytotoxicity, and tumor-protective immunity to melanoma were measured following three biweekly immunizations. The adjuvant potentiated the %antibody% response to some, but not all, melanoma antigens in a dose-dependent fashion. The adjuvant also potentiated cellular immunity as measured by in vitro cytotoxicity assays. No potentiation of tumor-protective immunity was detected. In comparison to Freund's complete adjuvant, cell wall skeleton plus monophosphoryl lipid A (CWS:%MPL%) induced fewer cutaneous toxic effects and stronger %antibody% and DTH responses but resulted in no greater in vitro cytotoxicity or tumor-protective immunity. Thus, the adjuvant had a selective and dose-dependent effect on humoral responses to vaccine immunization but did not potentiate a tumor-protective immunity to B16 melanoma.

7/3,AB/80 (Item 80 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06752331 91313830
Enhanced resistance to cattle grub infestation (Hypoderma lineatum de Vill.) in calves immunized with purified hypodermin A, B and C plus monophosphoryl lipid A (%MPL%).
Baron RW; Colwell DD
Agriculture Canada Research Station, Lethbridge, Alta, Canada.
Vet Parasitol (NETHERLANDS) Mar %1991%, 38 (2-3) p185-97, ISSN 0304-4017 Journal Code: XBU
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The influence of an antigen-specific cellular and humoral immune response, stimulated by immunization, on survival of a challenge infestation of Hypoderma lineatum was investigated. Calves immunized with a purified combination of hypodermin A, B and C plus monophosphoryl lipid A (%MPL%) developed a strong antigen-specific cellular immune response by completion of the immunization schedule which persisted to 12 weeks post-infestation. Responsiveness of peripheral blood lymphocytes to the mitogens concanavalin A and pokeweed was also elevated at 4 and 12 weeks post-infestation. Western blot analysis at the time of maximum grub counts demonstrated that immunized calves responded to hypodermin A, B and C while those receiving only %MPL% or infested controls responded only to hypodermin B and C. The antigen-specific %antibody% response as measured by ELISA at

maximum grub count was significantly higher in vaccinated calves than in infested controls while the response in calves receiving only immunostimulator was also significantly elevated. Immunized (antigen plus %MPL%) calves produced 5.0 +/- 6.9 grubs per animal which successfully pupated while those receiving %MPL% alone produced 16.4 +/- 6.1 and infested controls produced 32.2 +/- 10.9 grubs per animal.

7/3,AB/81 (Item 81 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06733822 91281133
Syphilis vaccine: up-regulation of immunogenicity by cyclophosphamide, Ribi adjuvant, and indomethacin confers significant protection against challenge infection in rabbits.
Fitzgerald TJ
Department of Medical Microbiology and Immunology, University of Minnesota School of Medicine, Duluth 55812.
Vaccine (ENGLAND) Apr %1991%, 9 (4) p266-72, ISSN 0264-410X Journal Code: X60
Contract/Grant No.: AI 18619, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Many unsuccessful attempts have been made to develop effective vaccines against experimental syphilitic infection. The focus of this report was to evaluate newer approaches to up-regulate immune responses following immunization with Treponema pallidum. Rabbits were injected once on day 0 with heat-inactivated treponemes suspended in the Ribi adjuvant system containing monophosphoryl lipid A (%MPL%) and trehalose dimycolate; animals were challenged dermally on day 29 with viable organisms. Various up-regulating agents were then tested using this general immunization protocol. When rabbits were pretreated on day -2 with cyclophosphamide (CYC), no protection was apparent. CYC pretreatment exhibited some protection when combined with a daily course of indomethacin on days 29 to 36. When rabbits were injected on day 0, then given a boost of %MPL% alone on day +2 plus indomethacin on days 29 to 36, minor protection was again apparent. Excellent protection was achieved when the vaccine protocol involved a combination of CYC pretreatment on day -2, an %MPL% boost on day +2, and indomethacin on days 29 to 36. Ninety-two percent of the subsequent lesions were atypical as indicated by their flat appearance, small size, lack of ulceration, and rapid healing. Importantly, this vaccine regimen also decreased dissemination of T. pallidum to distant tissues. These results suggest a new perspective in understanding immune responses in syphilis. We propose that vaccination, like infection, generates immune down-regulation that counter-balances immune stimulation. THus, effective vaccines will depend on removal and/or neutralization of treponemal components that down-regulate immune reactivity.

7/3,AB/82 (Item 82 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06655289 90170131
Enrichment of suppressor T cells by means of binding to monophosphoryl lipid A.
Baker PJ; Haslov KR; Fauntleroy MB; Stashak PW; Myers K; Ulrich JT Laboratory of Immunogenetics, Twinbrook-II Research Facility, National Institute of Allergy and Infectious Diseases, Rockville, Maryland 20852. Infect Immun (UNITED STATES) Mar %1990%, 58 (3) p726-31, ISSN 0019-9567 Journal Code: GO7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The binding and elution of spleen cells from plastic dishes coated with monophosphoryl lipid A (%MPL%) resulted in a greater than 1,000-fold enrichment of antigen-specific suppressor T-cell (TS) activity when spleen cells from mice 18 to 24 h after exposure to a low dose of type III pneumonococcal polysaccharide (SSS-III) were used. The removal of %MPL% -adherent TS cells resulted in an increase in the degree of amplifier T-cell (TA) activity present in the remaining %MPL%-nonadherent cell fraction; however, both TS and TA activities were found in the %MPL% -adherent cell fraction when spleen cells from mice 4 days after immunization with an optimal dose of SSS-III were examined. These findings, as well as others, suggest that both TS and TA, once activated, acquire a cell surface receptor that enables them to bind to %MPL%. Because of differences in the kinetics for the activation of TS and TA during the course of the %antibody% response and the fact that TS, but not TA, activity appears as early as 18 to 24 h after exposure to SSS-III, it is possible to use this experimental approach to obtain cell suspensions greatly enriched in TS activity.

7/3,AB/83 (Item 83 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06621397 90354047
Inactivation of suppressor T cell activity by the nontoxic lipopolysaccharide of Rhodopseudomonas sphaeroides.
Baker PJ; Taylor CE; Stashak PW; Fauntleroy MB;

Haslov K; Qureshi N; Takayama K
Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, Rockville, Maryland 20852.
Infect Immun (UNITED STATES) Sep %1990%, 58 (9) p2862-8, ISSN 0019-9567 Journal Code: GO7
Contract/Grant No.: GM-36054, GM, NIGMS; AI-25856, AI, NIAID Languages: ENGLISH
Document type: JOURNAL ARTICLE
%Antibody% responses of mice immunized with type III pneumococcal polysaccharide were examined with and without treatment with nontoxic lipopolysaccharide from Rhodopseudomonas sphaeroides (Rs-LPS). The results obtained were similar to those described previously for mice treated with monophosphoryl lipid A (%MPL%) except that lower amounts of Rs-LPS were needed. Both were without effect when given at the time of immunization with type III pneumococcal polysaccharide but elicited significant enhancement when given 2 to 3 days later. Such enhancement was T cell dependent and not due to polyclonal activation of immunoglobulin M synthesis by B cells. Treatment with either Rs-LPS or %MPL% abolished the expression but not induction of low-dose paralysis, a form of immunological unresponsiveness known to be mediated by suppressor T cells (Ts). The in vitro treatment of cell suspensions containing Ts with extremely small amounts of Rs-LPS or MPI completely eliminated the capacity of such cells to transfer suppression to other mice. These findings indicate that the immunomodulatory effects of both %MPL% and Rs-LPS are mainly the result of eliminating the inhibitors effects of Ts; this permits the positive effects of amplifier T cells to be more fully expressed, thereby resulting in an increased %antibody% response. The significance of these and other findings to the use of Rs-LPS as a pharmacotherapeutic agent for gram-negative bacterial sepsis is discussed.

7/3,AB/84 (Item 84 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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06488093 91012363
The effect of adjuvants on %antibody% titers in mouse vaginal fluid after intravaginal immunization.
Thapar MA; Parr EL; Parr MB
Department of Anatomy, School of Medicine, Southern Illinois University, Carbondale 62901.
J Reprod Immunol (NETHERLANDS) Jun %1990%, 17 (3) p207-16, ISSN 0165-0378 Journal Code: JWS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Intravaginal (ivag) immunization elicits secretory immune responses in the female reproductive tract, but little is known about the safety and effectiveness of adjuvants for such immunization. Mice were immunized intravaginally once daily for 5 days with large doses of horse ferritin combined with aluminum hydroxide (AH), muramyl dipeptide (MDP), monophosphoryl lipid A (%MPL%), dimethyl dioctadecyl ammonium bromide (DDA) or cholera toxin (CT). Titers of anti-ferritin IgA and IgG were measured in vaginal fluid by ELISA. The most effective adjuvant for ivag primary immunization was AH, while %MPL% was most effective for ivag boosting. None of the adjuvants caused a detectable tissue reaction in vaginal mucosa. Primary ivag immunization for 5 days with ferritin and AH followed by ivag boosting for 5 days with ferritin and %MPL% elicited higher IgA titers in vaginal fluid than systemic priming and boosting with ferritin and AH or systemic priming and ivag boosting with ferritin and %MPL%. Systemically immunized animals exhibited the highest IgG titers in vaginal fluid. The data indicate that adjuvants, particularly AH, can increase local immune responses to intravaginal immunization, but it should be noted that multiple applications of large doses of antigen were used and that this route of sensitization may be relatively inefficient.

7/3,AB/85 (Item 85 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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06461539 90224758
A study of the cellular and molecular mediators of the adjuvant action of a nontoxic monophosphoryl lipid A.
Johnson AG; Tomai MA
Department of Microbiology/Immunology, School of Medicine, University of Minnesota, Duluth 55812.
Adv Exp Med Biol (UNITED STATES) %1990%, 256 p567-79, ISSN 0065-2598 Journal Code: 2LU
Contract/Grant No.: AI 25810, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
A detoxified endotoxin, termed monophosphoryl lipid A (%MPL%, Ribi), has been shown to increase %antibody% forming cell numbers in aging Balb mice both in vivo and in vitro. Separation of splenocytes from aging mice into purified T, B and adherent cell populations and subsequent incubation of each with %MPL% and admixture with their cellular counterparts and antigen, revealed only the T cell compartment capable of transferring the adjuvant action. Incubation of purified T cells from aging mice with %MPL% for 2 hr, followed by washing and culture for 48 hr, resulted in a supernatant fluid which enhanced %antibody% formation in cultures of aging spleen cells. This enhancing action was eliminated by an antiserum containing anti-alpha/beta/gamma interferon, but not by an anti-alpha/beta interferon antiserum. These data, as well as evidence gained by others as discussed, suggests the hypothesis wherein

%MPL% increases %antibody% formation in aging mice by inducing the helper T cell population to secrete interferon gamma. The latter activates the macrophage to secrete increased levels of interleukin 1, thereby resulting in increased responsiveness throughout the ensuing sequence of cellular and molecular events leading to %antibody% synthesis.

7/3,AB/86 (Item 86 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06186519 86277093
Immunobiological activities of nontoxic lipid A: enhancement of nonspecific resistance in combination with trehalose dimycolate against viral infection and adjuvant effects.
Masihi KN; Lange W; Brehmer W; Ribi E
Int J Immunopharmacol (ENGLAND) %1986%, 8 (3) p339-45, ISSN 0192-0561 Journal Code: GRI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The ability of nontoxic monophosphoryl lipid A (%MPL%) to stimulate nonspecific resistance against viral infection was investigated. Mice pretreated intravenously with squalane-in-water emulsions of %MPL%, alone or in combination with other immunostimulants, were given an aerosol of influenza virus three weeks after the pretreatment. Complete protection against lethal influenza virus infection was conferred when %MPL% was combined with trehalose dimycolate (TDM). The protective activity of %MPL% plus TDM combination was corroborated by a significant reduction of the lung virus titers. Combination of lower doses of %MPL% with TDM extracted from Mycobacterium bovis, but not with that of M. phlei, induced significant resistance to influenza virus. Preparations containing %MPL% alone, or combined with mycobacterial cell wall skeleton or muramyl dipeptide, were not effective. The adjuvant activity of %MPL% on bivalent influenza subunit vaccine was also studied. The primary %antibody% responses to influenza A and influenza B antigens were enhanced by the addition of %MPL% and were higher than the vaccine associated with aluminum hydroxide. The adjuvant activity of %MPL% was confirmed by the elevated secondary response. High levels of circulating %antibodies% were still present in the %MPL% group when %antibody% titers in the controls were waning.

7/3,AB/87 (Item 87 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06111656 87190156
Purification and partial characterization of two prolactin-like glycoprotein hormone complexes from the midpregnant mouse conceptus. Colosi P; Ogren L; Thordarson G; Talamantes F
Endocrinology (UNITED STATES) Jun %1987%, 120 (6) p2500-11, ISSN 0013-7227 Journal Code: EGZ
Contract/Grant No.: HD-14966, HD, NICHD; RR-08132, RR, NCRR Languages: ENGLISH
Document type: JOURNAL ARTICLE
Two PRL-like glycoprotein hormone complexes were purified from the medium of cultured mouse conceptuses from day 10 of pregnancy: mouse placental lactogen-I (%mPL%-I) (29-32K), and %mPL% -I (36.5-42K). Sodium dodecyl sulfate-gel electrophoresis revealed that %mPL%-I (36.5-42K) is a complex of five proteins with mol wt of 36.5K, 37.5K, 39K, 40.5K, and 42K. Deglycosylation with peptide: N-glycosidase F or trifluoromethanesulfonic acid produced a single 29K protein. %mPL%-I (36.5-42K) was also sensitive to neuraminidase, but not to endo-beta-N-acetylglucosaminidase H or bacterial alkaline phosphatase. The production of intermediates from partial digestion of %mPL%-I (36.5-42K) with endo-beta-N-acetylglucosaminid ase F indicated the presence of multiple glycosylation sites. %mPL%-I (29-32K) is a complex of three proteins with mol wt of 29K, 30.5K, and 32K. Treatment with peptide:N-glycosidase F or trifluoromethanesulfonic acid reduced the mol wt of the 30.5K and 32K bands to 28K. The 30.5K band was sensitive to endo-beta-N-acetylglucosaminidase H and endo-beta-N-acetylgluc osaminidase F, but the 32K band was not. Neither band was sensitive to neuraminidase or bacterial alkaline phosphatase. The 29K band was resistant to all chemical and enzymatic treatments and is probably not glycosylated or phosphorylated. In the nonreduced state, neither form of %mPL%-I showed an increase in mobility over that of its reduced counterpart on sodium dodecyl sulfate-gel electrophoresis, indicating that neither form of %mPL% -I contains the large disulfide loop common to hormones of the PRL family. After iodination, all component proteins of both forms of %mPL%-I were found to bind to day 17 pregnant mouse liver membranes and were displaceable by excess %mPL%-II. In a radioreceptor assay, 125I-labeled %mPL%-I (36.5-42K) was displaced by mPRL or %mPL%-II, but not by mGH. An antiserum to both forms of %mPL%-I was generated, and a RIA employing %mPL% -I (36.5-42K) as the standard and radioligand was developed. Dilutions of day 10 pregnant maternal mouse serum and placental homogenate and a partially purified fraction of %mPL% -I (29-32K) produced displacement curves parallel to that of %mPL% -I (36.5-42K) standard curve. Five micrograms of mPRL, %mPL%-II, or mGH or 10 microliter day 17 pregnant or male mouse serum did not displace the

radioligand from the %antibody%. %mPL% -I (36.5-42K) was lactogenic, but it did not possess LH-like bioactivity.

7/3,AB/88 (Item 88 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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06054475 88186173
Inactivation of suppressor T-cell activity by nontoxic monophosphoryl lipid A.
Baker PJ; Hiernaux JR; Fauntleroy MB; Prescott B; Cantrell JL; Rudbach JA Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.
Infect Immun (UNITED STATES) May %1988%, 56 (5) p1076-83, ISSN 0019-9567 Journal Code: GO7
Contract/Grant No.: 263-80-D-0394
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Treatment with nontoxic monophosphoryl lipid A (%MPL%), which was derived from a polysaccharide-deficient, heptoseless Re mutant of Salmonella typhimurium, was found to inactivate suppressor T-cell activity, as evidenced by a decrease in the degree of low-dose immunological paralysis expressed and an increase in the magnitude of the %antibody% response to type III pneumococcal polysaccharide. The effects produced, which could not be attributed to the polyclonal activation of immune B cells by %MPL%, were dependent upon the dose of %MPL% used, as well as the time when %MPL% was given relative to low-dose priming or immunization with type III pneumococcal polysaccharide. Neither amplifier nor helper T-cell activity was decreased by treatment with the same, or larger, doses of %MPL%. The significance of these findings to the use of %MPL% as an immunological adjuvant or an immunomodulating agent is discussed.

7/3,AB/89 (Item 89 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

05779397 90095482
T cell and interferon-gamma involvement in the adjuvant action of a detoxified endotoxin.
Tomai MA; Johnson AG
Department of Medical Microbiology/Immunology, University of Minnesota, Duluth 55812.
J Biol Response Mod (UNITED STATES) Dec %1989%, 8 (6) p625-43, ISSN 0732-6580 Journal Code: JBM
Contract/Grant No.: AI 25810, AI, NIAID; AG 06198, AG, NIA Languages: ENGLISH
Document type: JOURNAL ARTICLE
The adjuvant activities of a detoxified derivative of endotoxic lipopolysaccharides, isolated from the outer membrane of gram-negative bacteria, were evaluated in aging mice. This monophosphoryl lipid A (%MPL%) (Ribi) was capable of enhancing %antibody% production in vitro in splenic cultures from 2-3-month-old male Balb/c mice as well as cultures from 22-24-month-old Balb/c mice. Separation of spleen cells from %MPL% and phosphate-buffered saline-injected mice into adherent and nonadherent populations and subsequent mixing of populations and culture with antigen implicated an adherent cell as being involved in the enhancement of %antibody% formation induced by %MPL%. However, separation of normal spleen cells into purified populations of adherent cells, T-lymphocytes, and B-lymphocytes, followed by in vitro stimulation of the individual populations with %MPL% and subsequent transfer into cultures of normal spleen cells, revealed only the T cell as capable of transferring the enhancement of %antibody% formation. In addition, culture filtrates from %MPL%-stimulated T cells were able to enhance %antibody% production by spleen cell cultures from aging mice twofold above that of filtrates from unstimulated T cells. The enhancement of %antibody% formation induced by such filtrates and also by %MPL% in spleen cell cultures from young and aging mice was inhibited by a monoclonal %antibody% (MAb) to recombinant interferon-gamma (rIFN-gamma) as well as antiserum against IFN-alpha, -beta, and -gamma, but not by an antiserum to IFN-alpha/beta. Enhancement of %antibody% formation correlated well with an increase in interleukin-1 (IL-1) but not with an increase in IL-2 production. Addition of anti-asialo-GM1 MAb plus complement to the effective spleen populations did not diminish the adjuvant action.

7/3,AB/90 (Item 90 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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05614904 89138670
Adjuvant effects of trehalose dimycolate on the %antibody% response to type III pneumococcal polysaccharide.
Baker PJ; Fauntleroy MB; Stashak PW; Hiernaux JR; Cantrell JL; Rudbach JA National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.
Infect Immun (UNITED STATES) Mar %1989%, 57 (3) p912-7, ISSN 0019-9567 Journal Code: GO7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Treatment with trehalose dimycolate (TDM) increases the magnitude of the immunoglobulin M (IgM) %antibody%

response of mice to type III pneumococcal polysaccharide (SSS-III). Such enhancement is demonstrable over a wide range of immunizing doses and does not require thymus-derived (T) cells to be elicited. Although young adult mice immunized with SSS-III do not usually make anti-SSS-III %antibodies% of the IgG1 and IgG3 classes, %antibodies% of one or both isotypes were produced after immunization and treatment with TDM and/or monophosphoryl lipid A (%MPL%); the additive nature of the effect produced by both TDM and %MPL% suggests that the two immunomodulators act by different mechanisms. TDM and %MPL% have different effects on the induction and expression of low-dose immunological paralysis, a form of unresponsiveness known to be mediated by suppressor T cells. The relevance of these findings to the modes of action of TDM and %MPL% is discussed.

7/3,AB/91 (Item 91 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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05545823 89212896
Immunomodulatory activity of monophosphoryl lipid A in C3H/HeJ and C3H/HeSnJ mice.
Hiernaux JR; Stashak PW; Cantrell JL; Rudbach JA; Baker PJ Ribi ImmunoChem Research, Inc., Hamilton, Montana 59840. Infect Immun (UNITED STATES) May %1989%, 57 (5) p1483-90, ISSN 0019-9567 Journal Code: GO7
Contract/Grant No.: 263-80-D-0394
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Treatment with nontoxic monophosphoryl lipid A (%MPL%) derived from a polysaccharide-deficient, heptoseless Re mutant of either Salmonella typhimurium or Salmonella minnesota R595 enhanced the immunoglobulin M (IgM) anti-type III pneumococcal polysaccharide (SSS-III) %antibody% response of C3H/HeSnJ mice. Such an adjuvant effect was not observed in lipopolysaccharide-nonresponder C3H/HeJ mice. Nevertheless, C3H/HeJ spleen cells produced a weak mitogenic response to both preparations of %MPL% in vitro, and C3H/HeJ mice showed a significant increase in serum IgM levels without an increase in numbers of splenic IgM-secreting plaque-forming cells after in vivo treatment with %MPL%. A significant increase in serum IgG3 levels was accompanied by a transient decrease in serum IgG1 levels in C3H/HeSnJ mice given %MPL%; such non-antigen-specific polyclonal effects were not observed in C3H/HeJ or in athymic nu/nu mice. Since the enhanced %antibody% response to SSS-III has been attributed to the inactivation of suppressor T cells by %MPL% and since suppressor-T-cell activity is demonstrable in both C3H/HeSnJ and C3H/HeJ mice, these findings imply that (i) the suppressor T cells of C3H/HeJ mice are refractory to inactivation by %MPL% and (ii) some of the polyclonal and mitogenic effects produced in C3H/HeJ mice are due to the direct action of %MPL% on B lymphocytes.

7/3,AB/92 (Item 92 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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05506454 88090858
Immunoprotective behaviour of plasma-membrane-associated antigens of axenic Entamoeba histolytica.
Vinayak VK; Purnima; Saxena A
Department of Experimental Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh, India.
J Med Microbiol (ENGLAND) Dec %1987%, 24 (4) p297-302, ISSN 0022-2615 Journal Code: J2N
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Immunisation of golden hamsters with plasma-membrane-associated antigens of a virulent subline of axenic Entamoeba histolytica strain NIH 200 V, entrapped in multilamellar phosphatidylcholine liposomes (%MPL%) or Freund's complete adjuvant (FCA), afforded protection against intrahepatic challenge with axenic amoebic trophozoites of the same strain. Amoebic liver abscess developed in 86% and 80% of the animals that received empty liposomes or buffer emulsified in FCA but in none of the animals that received plasma-membrane-antigen vaccines. All the immunised animals had significantly higher levels (p less than 0.001) of %antibodies% to plasma-membrane components and significantly higher levels (p less than 0.001) of cellular sensitisation. %Antibody%-dependent macrophage-mediated cytotoxicity against trophozoites was also found to be significantly greater (p less than 0.001) in immunised animals. Liposome-entrapped antigens stimulated the immune system of the host as well as, or better than, antigens administered with FCA.

7/3,AB/93 (Item 93 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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05052607 87225056
The adjuvant properties of a nontoxic monophosphoryl lipid A in hyporesponsive and aging mice.
Tomai MA; Solem LE; Johnson AG; Ribi E
J Biol Response Mod (UNITED STATES) Apr %1987%, 6 (2) p99-107, ISSN 0732-6580 Journal Code: JBM
Languages: ENGLISH

Document type: JOURNAL ARTICLE
The immunomodulatory action of a nontoxic monophosphoryl lipid A (%MPL%) and a toxic diphosphoryl lipid A (DPL) fraction derived from endotoxins of the heptoseless mutants of bacteria were studied. Both derivatives retained the ability characteristic of lipopolysaccharides, i.e., to enhance %antibody% formation in young adult mice when injected along with antigen and suppress %antibody% production when given 1 day before antigen. In aging mice, a model of immunodeficiency, a marked restoration of %antibody% formation was observed when antigen was injected together with either %MPL% or DPL. Levels of %antibody% in the aging mice became comparable with those observed in young adult mice. Moreover, both %MPL% and DPL enhanced %antibody% production significantly in the endotoxin low-responder mouse strains C3H/HeJ and C57Bl/10 ScN, whereas phenol-water-extracted endotoxin from an Rd mutant was ineffective. %MPL% and DPL also acted as suppressive agents when administered prior to antigen in the C3H/HeJ strain. Thus, the results from these studies show that the toxic properties of lipid A can be removed without eliminating immunomodulating activity and certain forms of lipid A can overcome the immunologic lesions of immunodeficient and hyporesponsive animals.

7/3,AB/94 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06337894 Genuine Article#: YK462 Number of References: 31 Title: Megakaryocytes derived from CD34-positive cord blood cells produce interleukin-8
Author(s): Higuchi T; Koike K (REPRINT) ; Sawai N; Mwamtemi HH; Takeuchi K; Shiohara M; Kikuchi T; Yasui K; Ito S; Yamagami O; Sasaki Y; Okumura N; Kato T; Miyazaki H; Ikeda M; Yamada M; Komiyama A
Corporate Source: SHINSHU UNIV,SCH MED, DEPT PAEDIAT, 3-1-1 ASAHI/MATSUMOTO/NAGANO 390/JAPAN/ (REPRINT); SHINSHU UNIV,SCH MED, DEPT PAEDIAT/MATSUMOTO/NAGANO 390/JAPAN/; SHINSHU UNIV HOSP,BLOOD TRANSFUS SERV/MATSUMOTO/NAGANO/JAPAN/; SHINSHU UNIV HOSP,CENT CLIN LABS/MATSUMOTO/NAGANO/JAPAN/; SHINSHU UNIV,SCH ALLIED MED SCI, DIV CLIN CHEM & MED TECHNOL, SCH MED/MATSUMOTO/NAGANO 390/JAPAN/; KIRIN BREWERY CO LTD,PHARMACEUT RES LAB/MAEBASHI/GUMMA 371/JAPAN/; MORINAGA MILK IND CO LTD,BIOCHEM RES LAB/KANAGAWA//JAPAN/
Journal: BRITISH JOURNAL OF HAEMATOLOGY, %1997%, V99, N3 (DEC), P509-516 ISSN: 0007-1048
Publication date: 19971200
Publisher: BLACKWELL SCIENCE LTD, OSNEY MEAD, OXFORD, OXON, ENGLAND OX2 0EL Language: English
Document Type: ARTICLE
Abstract: In a serum-free liquid culture, thrombopoietin (TPO) selectively stimulated the growth of megakaryocytic cells from CD34-positive cord blood cells. Using these cultured cells, we investigated cytokine production by human megakaryocytes, Day 10 megakaryocytes (2 x 10(5)) secreted > 1000 pg/ml of interleukin (IL)-8, in contrast to small amounts of IL-1 beta and IL-6. A time-course study showed that the IL-8 production of megakaryocytes occurred at the late phase of the culture period. The megakaryocyte-conditioned medium had the chemotactic potential of polymorphonuclear leucocytes, which was abrogated by the addition of anti-IL-8 %antibody%, suggesting the secretion of biologically active IL-8. The combination of TPO and IL-1 alpha was required for a significant augmentation of the IL-8 secretion, Direct evidence for IL-8 synthesis in megakaryocytes was provided by reverse transcription-polymerase chain reaction on purified CD41b(+) cells and by the detection of intracellular IL-8 in CD41b(+) cells, These results suggest that TPO stimulates not only the proliferation and differentiation of the progenitors capable of megakaryocytic lineage expression but also IL-S release by the megakaryocytic cells with the aid of IL-1.

7/3,AB/95 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06303948 Genuine Article#: YG425 Number of References: 0 Title: Inhibition of biological activity of thrombopoietin by monoclonal %antibodies% to human c-%Mpl%.
Author(s): Ohashi H; Morita H; Tahara T; Tsunakawa H; Matsumoto A; Ogami K; Oda A; Ikeda Y; Miyazaki H; Kato T
Corporate Source: KEIO UNIV,DEPT INTERNAL MED, DIV HEMATOL/TOKYO//JAPAN/; KIRIN BREWERY CO LTD,PHARMACEUT RES LAB/GUNMA//JAPAN/
Journal: BLOOD, %1997%, V90, N10,2,1 (NOV 15), P3533-3533
ISSN: 0006-4971 Publication date: 19971115
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399
Language: English Document Type: MEETING ABSTRACT

7/3,AB/96 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

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06265920 Genuine Article#: YF299 Number of References: 41 Title: Recombinant human interleukin-11 directly promotes
megakaryocytopoiesis in vitro
Author(s): Weich NS (REPRINT) ; Wang AL; Fitzgerald M; Neben TY; Donaldson D; Giannotti J; YetzAldape J; Leven RM; Turner KJ
Corporate Source: GENET INST INC,DEPT IMMUNOL & HEMATOPOIESIS, 87 CAMBRIDGE PK DR/CAMBRIDGE//MA/02140 (REPRINT); RUSH MED COLL,DEPT ANAT/CHICAGO//IL/60612
Journal: BLOOD, %1997%, V90, N10 (NOV 15), P3893-3902
ISSN: 0006-4971 Publication date: 19971115
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399
Language: English Document Type: ARTICLE
Abstract: We have investigated the mechanism of action of the thrombopoietic cytokine, recombinant human interleukin-11 (rhIL-17), on megakaryocytopoiesis in vitro. We have shown that rhIL-11-induced murine and human megakaryocytopoiesis are not mediated by thrombopoietin (Tpo). Murine megakaryocytes (MKs) were produced from bone marrow (BM) mononuclear cells cultured with rhIL-11, IL-3, and a combination of the two cytokines. Conditioned media (CM) were collected and assayed for the presence of biologically active Tpo. Tpo activity was not detected in any of the CMs tested. Next, human BM CD34(+) cells were cultured in serum-free fibrin clot medium with rhIL-11, IL-3, or rhIL-11 plus IL-3 and an %antibody% that neutralizes human Tpo activity. No inhibition of either burst-forming unit-MK- or colony-forming unit-MK-derived colony formation was observed. The %antibody% did partially inhibit steel factor-induced MK-colony formation, suggesting that the actions of this cytokine are mediated, in part, by Tpo. We determined that MKs can be direct targets of rhIL-11 by showing the expression of functional IL-11 receptor on these cells. Total RNA was prepared from cultured human BM CD41(+)CD14(-) cells (MKs) and IL-11 receptor alpha chain mRNA was detected in the MKs by reverse transcription-polymerase chain reaction. Analysis of single-sorted CD41(+)CD14(-) cells confirmed that the observed IL-11 receptor expression was not due to contaminating CD41(-) cells in the pool. The presence of rhIL-11 receptor alpha chain protein in the cells was established by Western blot analysis. After a short exposure of purified BM MKs to rhIL-11, enhanced phosphorylation of both its signal transduction subunit, gp130, and the transcription factor, STAT3 was detected, showing a direct activation of receptor signaling by the cytokine. Consistent with the lack of effect of rhIL-11 on platelets in vivo, IL-11 receptor alpha chain mRNA and protein were not detected in isolated human platelets. These data indicate that rhIL-11 acts directly on MKs and MK progenitors but not on platelets. (C) 1997 by The American Society of Hematology.

7/3,AB/97 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06259250 Genuine Article#: YE776 Number of References: 56 Title: Blood thrombopoietin, IL-6 and IL-11 levels in patients with agnogenic myeloid metaplasia
Author(s): Wang JC (REPRINT) ; Chen C; Lou LH; Mora M
Corporate Source: BROOKDALE UNIV HOSP & MED CTR,DIV HEMATOL ONCOL/BROOKLYN//NY/11212 (REPRINT); BROOKDALE UNIV HOSP & MED CTR,DIV MED ONCOL & HEMATOL/BROOKLYN//NY/; BROOKDALE UNIV HOSP & MED CTR,DEPT PATHOL/BROOKLYN//NY/
Journal: LEUKEMIA, %1997%, V11, N11 (NOV), P1827-1832
ISSN: 0887-6924 Publication date: 19971100
Publisher: STOCKTON PRESS, HOUNDMILLS, BASINGSTOKE, HAMPSHIRE, ENGLAND RG21 6XS
Language: English Document Type: ARTICLE
Abstract: Agnogenic myeloid metaplasia (AMM) is a disease characterized by bone marrow megakaryocyte hyperplasia and clusters of megakaryocytes, in which many of the megakaryocytes are atypical. In order to elucidate the mechanisms of megakaryocytosis, ELISA assays of blood levels of thrombopoietin (TPO), interleukin-6 (IL-6) and interleukin-ll (IL-ll) were done in 45 patients with AMM and compared with normal volunteer controls. Higher blood TPO levels were found in AMM than in controls (P < 0.0001), and blood TPO levels were correlated with the degree of marrow fibrosis (P = 0.0078). Blood levels of IL-6 were also significantly higher in AMM, when compared with controls (P<0.0001), However, no correlation was found between blood IL-6 levels and degree of marrow fibrosis. No correlation was found between either TPO or IL-6 and the number of blood platelet counts, the number of marrow megakaryocytes, WBC counts, or the degree of splenomegaly, Blood IL-11 levels were undetectable in most patients and no significant difference was found in AMM as compared to controls, The present study demonstrated that, while in idiopathic thrombocytopenic purpura (ITP) or aplastic anemia, blood TPO levels are relatively correlated with the numbers of platelet and/or megakaryocyte mass, blood TPO levels do not correlate with blood platelet counts, or marrow megakaryocyte mass in AMM, Therefore, in AMM,

other mechanisms such as the number of TPO receptors on platelets or megakaryocytes, c-%MPL% receptor abnormalities, abnormal production of TPO mRNA and so on, will have to be studied, Furthermore, TPO may play a significant role in the pathogenesis of marrow fibrosis; IL-6 may be a factor in the development of marrow megakaryocytosis but its elevated blood levels may represent a secondary immune phenomenon; and IL-ll probably does not play a significant role in causing marrow megakaryocytosis in this disease.

7/3,AB/98 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06207897 Genuine Article#: YB756 Number of References: 44 Title: Augmentation of natural antiganglioside IgM %antibodies% in lower motor neuron disease (LMND) and role of CD5+ B cells Author(s): Ravindranath RMH; Ravindranath MH (REPRINT) ; Graves MC Corporate Source: JOHN WAYNE INST CANC TREATMENT & RES,2200 SANTA MONICA BLVD/SANTA MONICA//CA/90404 (REPRINT); JOHN WAYNE INST CANC TREATMENT & RES,/SANTA MONICA//CA/90404; UNIV CALIF LOS ANGELES,REED NEUROL RES LAB, DEPT NEUROL/LOS ANGELES//CA/90024
Journal: CELLULAR AND MOLECULAR LIFE SCIENCES, %1997%, V53, N9 (SEP), P 750-758
ISSN: 1420-682X Publication date: 19970900
Publisher: BIRKHAUSER VERLAG AG, PO BOX 133 KLOSTERBERG 23, CH-4010 BASEL, SWITZERLAND
Language: English Document Type: ARTICLE
Abstract: IgM %antibodies% directed against neuronal gangliosides GM(1), GM(2), GD(1a), GD(1b) and GT(1b) occur in normal individuals and their level significantly decreases with age. Patients with lower motor neuron disease (LMND) produce high levels of these auto-%antibodies%. AntiGM(1) IgM is selectively augmented. In these patients, the CD5+ (B1) and CD5- (B2) subsets of B cells are not distinct entities but range from those without detectable CD5 marker to those with high CD5+ expression. B1 B cells were sorted to homogeneity, but B2 B cell cannot be isolated to homogeneity because of the presence of B1 cells with low CD5 expression. In short term cultures both the subsets produced IgM %antibodies%, but the %antibodies% reacted better with desialylated GM(1) than with GM(1). Cycloheximide (Cx) (0.35 mM) largely blocked IgM synthesis of the B1 B cells but inhibition of the B2 B cells was incomplete, possibly due to shedding of cytophilic %antibodies% as well as to the presence of B1 phenotype with loss of CD5 expression. CD5+ B cells may be involved in the production elf antiglycolipid IgM.

7/3,AB/99 (Item 6 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06196001 Genuine Article#: YA721 Number of References: 53 Title: Genomic organization and molecular characterization of a gene encoding HsPXF, a human peroxisomal farnesylated protein Author(s): Kammerer S (REPRINT) ; Arnold N; Gutensohn W; Mewes HW; Kunau WH ; Hofler G; Roscher AA; Braun A
Corporate Source: UNIV MUNICH,DR VON HAUNERSCHEN KINDERSPITAL, MOL BIOL LAB, LINDWURMSTR 4/D-80337 MUNICH//GERMANY/ (REPRINT); UNIV MUNICH,INST ANTHROPOL & HUMAN GENET/D-80336 MUNICH//GERMANY/; MAX PLANCK INST BIOCHEM,/D-82152 MARTINSRIED//GERMANY/; RUHR UNIV BOCHUM,INST PHYSIOL/D-44801 BOCHUM//GERMANY/; GRAZ UNIV,INST PATHOL/A-8036 GRAZ//AUSTRIA/
Journal: GENOMICS, %1997%, V45, N1 (OCT 1), P200-210
ISSN: 0888-7543 Publication date: 19971001
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495
Language: English Document Type: ARTICLE
Abstract: A protein modification essential for the cellular sorting of many biologically relevant proteins is the covalent attachment of prenyl lipids by specific transferases. Isoprenylation is known to render protein domains hydrophobic, thereby facilitating the interaction with lipid bilayers and/or membrane proteins. The target for the modification with farnesyl groups is the COOH-terminal sequence CaaX. Among the variety of farnesylated proteins the only one reported so far to be located to peroxisomes is the 37-kDa peroxisomal farnesylated hamster protein PxF. Recently we published data on the cDNA of the human gene HK33 (A. Braun et at., 1994, Gene 146: 291-295), which was revealed to be the human ortholog of PxF and was consequently renamed HsPXF. The genomic structure, molecular characterization, and evolutionary conservation of HsPXF are described herein. The exact location of the gene was defined as chromosome 1q22. The gene spans a region of approximately 9 kb, containing eight exons and seven introns. The 5' upstream region showed two potential Spl-binding sites and an Alu repetitive sequence. Luciferase reporter activating capacity confirmed the presumed promoter activity of this region. On the transcriptional level, we detected four splice variants originating either from exon skipping or from alternative splicing events. For the HsPXF protein, a carboxyterminal farnesylation at cysteine residues was demonstrated. Through the use

of HsPXF-specific %antibodies%, the protein was shown to be attached to the outer surface of peroxisomes. This localization together with the similarity to a peroxisomal assembly protein from Saccharomyces cerevisiae suggests HsPXF is involved in the process of peroxisomal biogenesis or assembly. (C) 1997 Academic Press.

7/3,AB/100 (Item 7 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

06190586 Genuine Article#: YA269 Number of References: 26 Title: Sensitive measurement of thrombopoietin by a monoclonal %antibody% based sandwich enzyme-linked immunosorbent assay
Author(s): Folman CC; vondemBorne AEGK (REPRINT) ; Rensink IHJAM; Gerritsen W; vanderSchoot CE; deHaas M; Aarden L
Corporate Source: UNIV AMSTERDAM,ACAD MED CTR, NETHERLANDS CANC INST, DEPT HEMATOL, DIV INTERNAL MED, MEIBER/NL-1105 AZ AMSTERDAM//NETHERLANDS/ (REPRINT); NETHERLANDS RED CROSS,BLOOD TRANSFUS SERV, CENT LAB/AMSTERDAM//NETHERLANDS/; UNIV AMSTERDAM,EXPT & CLIN IMMUNOL LAB/AMSTERDAM//NETHERLANDS/; NETHERLANDS CANC INST,DIV IMMUNOL/NL-1066 CX AMSTERDAM//NETHERLANDS/; UNIV AMSTERDAM,ACAD MED CTR, NETHERLANDS CANC INST, DEPT HEMATOL, DIV INTERNAL MED/NL-1105 AZ
AMSTERDAM//NETHERLANDS/
Journal: THROMBOSIS AND HAEMOSTASIS, %1997%, V78, N4 (OCT), P1262-1267 ISSN: 0340-6245
Publication date: 19971000
Publisher: F K SCHATTAUER VERLAG GMBH, P O BOX 10 45 45, LENZHALDE 3, D-70040 STUTTGART, GERMANY
Language: English Document Type: ARTICLE
Abstract: In this report a sensitive enzyme-linked immunosorbent assay (ELISA) for the measurement of plasma thrombopoietin (Tpo) is described that is solely based on monoclonal %antibodies% (MoAbs). The assay has an intra and inter-assay variance of 5-7% and 7-13%, respectively. Native and recombinant human Tpo (rhTpo) were recognized equally well, no cross reactivity with other cytokines was found and rhTpo added to plasma and serum was completely recovered. With the ELISA, Tpo concentrations in EDTA-anticoagulated plasma of all controls (n = 193) could be determined, since the limit of detection (2 +/- 0.8 A.U./ml, mean +/- sd) was lower than the concentration found in controls (11 +/- 8 A.U./ml, mean +/- sd; 2.5th-97.5th percentile: 4-32 A.U./ml). Tpo levels in serum were on average 3.4 times higher than in plasma.

We showed in vivo that Tpo is bound by platelets, as in thrombocytopenic patients (n = 5) a platelet transfusion immediately led to a drop in plasma Tpo level, whereas in patients receiving chemotherapy the induced thrombocytopenia was followed by a rise in plasma Tpo levels.

In summary, these results indicate that this ELISA is a reliable tool for Tpo measurements and is applicable for large scale studies.

7/3,AB/101 (Item 8 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06190540 Genuine Article#: YA274 Number of References: 56 Title: Enhancement of megakaryocytopoiesis by Campath-1G-treated natural killer cells
Author(s): Nagler A (REPRINT) ; Condiotti R; Lubina A; Deutsch VR Corporate Source: HADASSAH UNIV HOSP,DEPT BONE MARROW TRANSPLANT, CANC IMMUNOBIOL RES LAB/IL-91120 JERUSALEM//ISRAEL/ (REPRINT); HADASSAH UNIV HOSP,DEPT HEMATOL/IL-91120 JERUSALEM//ISRAEL/
Journal: BONE MARROW TRANSPLANTATION, %1997%, V20, N7 (OCT), P525-531 ISSN: 0268-3369 Publication date: 19971000
Publisher: STOCKTON PRESS, HOUNDMILLS, BASINGSTOKE, HAMPSHIRE, ENGLAND RG21 6XS
Language: English Document Type: ARTICLE
Abstract: Campath-1G is a CD52 (rat IgG2b) moAb used in bone marrow transplantation (BMT) to prevent graft-versus-host disease (GVHD) by the elimination of T cells via %antibody%-dependent cell cytotoxicity (ADCC) in vivo. We have previously reported that Campath-1G induces T cell proliferation, activation, and production of cytokines which in turn causes an enhancement of megakaryocytopoiesis in vitro. In view of the fact that recent studies have indicated that natural killer (NK) cells may also be involved in the regulation of megakaryocytopoiesis, we undertook the study of the in vitro effect of Campath-1G-treated NK cells on the regulation of megakaryocytopoiesis. Early burst-forming BFU-MK and late colony-forming CFU-MK were grown from 2 x 10(5) peripheral blood non-adherent mononuclear cells (NAMC) in plasma clots in the presence of aplastic canine plasma (PICS-J) which was used as megakaryocyte colony-stimulating factor (MK-CSF). The first step in elucidating this series of events was to investigate the direct influence of NK cells on megakaryocytopoiesis. Co-culturing NK cells (>85% CD16(+)) with autologous NAMC at a ratio of 1:1 resulted in a significant increase in the proliferation of

CFU-MK and BFU-MK over NAMC cultured alone. This effect was further enhanced upon exposure of NK to Campath-1G (0.1-3 mu g/ml). To investigate the possible influence of soluble factors released from NK cells treated with Campath-1G on MK maturation, conditioned medium (CM) derived from Campath-1G-treated-enriched populations of NK cells was found to enhance MK progenitor growth. Our data demonstrate that resting and Campath-1G-treated NK may be involved in the immunomodulation of megakaryocytopoiesis.

7/3,AB/102 (Item 9 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06153890 Genuine Article#: XY523 Number of References: 61 Title: Characterization of 18 new mutations in COL7A1 in recessive dystrophic epidermolysis bullosa provides evidence for distinct molecular mechanisms underlying defective anchoring fibril formation Author(s): Hovnanian A (REPRINT) ; Rochat A; Bodemer C; Petit E; Rivers CA; Prost C; Fraitag S; Christiano AM; Uitto J; Lathrop M; Barrandon Y; deProst Y
Corporate Source: UNIV OXFORD,WELLCOME TRUST CTR HUMAN GENET, WINDMILL RD/OXFORD OX3 7BN//ENGLAND/ (REPRINT); ECOLE NORMALE SUPER,DEPT BIOL/F-75231 PARIS//FRANCE/; HOP NECKER ENFANTS MALAD,DEPT DERMATOL/PARIS//FRANCE/; HOP NECKER ENFANTS MALAD,DEPT HISTOL/PARIS//FRANCE/; HOP ST LOUIS,DEPT HISTOL/PARIS//FRANCE/; THOMAS JEFFERSON UNIV,JEFFERSON MED COLL, DEPT DERMATOL/PHILADELPHIA//PA/19107 ; THOMAS JEFFERSON UNIV,JEFFERSON MED COLL, DEPT CUTANEOUS BIOL/PHILADELPHIA//PA/19107; THOMAS JEFFERSON UNIV,JEFFERSON MED COLL, DEPT BIOCHEM/PHILADELPHIA//PA/19107; THOMAS JEFFERSON UNIV,JEFFERSON MED COLL, DEPT MOL PHARMACOL/PHILADELPHIA//PA/19107
Journal: AMERICAN JOURNAL OF HUMAN GENETICS, %1997%, V61, N3 (SEP), P 599-610
ISSN: 0002-9297 Publication date: 19970900
Publisher: UNIV CHICAGO PRESS, 5720 S WOODLAWN AVE, CHICAGO, IL 60637 Language: English Document Type: ARTICLE
Abstract: We have characterized 21 mutations in the type VII collagen gene (COL7A1) encoding the anchoring fibrils, 18 of which were not previously reported, in patients from 15 unrelated families with recessive dystrophic epidermolysis bullosa (RDEB). COL7A1 mutations in both alleles were identified by screening the 118 exons of COL7A1 and flanking intron regions. Fourteen mutations created premature termination codons (PTCs) and consisted of nonsense mutations, small insertions, deletions, and splice-site mutations. A further seven mutations predicted glycine or arginine substitutions in the collagenous domain of the molecule. Two mutations were found in more than one family reported in this study, and six of the seven missense mutations showed clustering within exons 72-74 next to the hinge region of the protein. Patients who were homozygous or compound heterozygotes for mutations leading to PTCs displayed both absence or drastic reduction of COL7A1 transcripts and undetectable type VII collagen protein in skin. In contrast, missense mutations were associated with clearly detectable COL7A1 transcripts and with normal or reduced expression of type VII collagen protein at the dermo/epidermal junction. Our results provide evidence for at least two distinct molecular mechanisms underlying defective anchoring fibril formation in RDEB: one involving PTCs leading to mRNA instability and absence of protein synthesis, the other implicating mis-sense mutations resulting in the synthesis of type VII collagen polypeptide with decreased stability and/or altered function. Genotype-phenotype correlations suggested that the nature and location of these mutations are important determinants of the disease phenotype and showed evidence for interfamilial phenotypic variability.

7/3,AB/103 (Item 10 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06144114 Genuine Article#: XX928 Number of References: 72 Title: Immunogenicity of membrane-bound gangliosides in viable whole-cell vaccines
Author(s): Ravindranath MH (REPRINT) ; Morton DL
Corporate Source: JOHN WAYNE CANC INST,ST JOHNS HLTH CTR, LAB GLYCOLIPID IMMUNOTHERAPY, 2200 SANTA MONICA BLVD/SANTA MONICA//CA/90404 (REPRINT)
Journal: CANCER INVESTIGATION, %1997%, V15, N5, P491-499
ISSN: 0735-7907 Publication date: 19970000
Publisher: MARCEL DEKKER INC, 270 MADISON AVE, NEW YORK, NY 10016 Language: English Document Type: ARTICLE

7/3,AB/104 (Item 11 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

06140277 Genuine Article#: XX545 Number of References: 36 Title: Thrombopoietin inhibits in vitro osteoclastogenesis from murine bone marrow cells
Author(s): Wakikawa T; Shioi A (REPRINT) ; Hino M;

Inaba M; Nishizawa Y; Tatsumi N; Morii H; Otani S
Corporate Source: OSAKA CITY UNIV,SCH MED, DEPT INTERNAL MED 2, ABENO KU, 1-5-7 ASAHI MACHI/OSAKA 545//JAPAN/ (REPRINT); OSAKA CITY UNIV,SCH MED, DEPT INTERNAL MED 2, ABENO KU/OSAKA 545//JAPAN/; OSAKA CITY UNIV,SCH MED, DEPT CLIN HEMATOL/OSAKA 545//JAPAN/
Journal: ENDOCRINOLOGY, %1997%, V138, N10 (OCT), P4160-4166 ISSN: 0013-7227 Publication date: 19971000
Publisher: ENDOCRINE SOC, 4350 EAST WEST HIGHWAY SUITE 500, BETHESDA, MD 20814-4110
Language: English Document Type: ARTICLE
Abstract: To determine whether thrombopoietin (TPO) can modulate the osteoclastic differentiation from hematopoietic stem cells, we investigated the effect of TPO on in vitro osteoclastogenesis by using the coculture of murine bone marrow cells with the stromal cell line (ST2) in the presence of 1 alpha,25-dihydroxyvitamin D-3 and dexamethasone. Recombinant human TPO inhibited the formation of tartrate-resistant acid phosphatase-positive multinucleated cells in a dose-dependent manner (0.02-200 ng/ml). The effect of TPO on differentiation of bone-resorbing capacity was investigated by pit assay. TPO dose dependently decreased the areas of toluidine blue-stained resorption pits (2.0-200 ng/ml). To identify the cellular target of TPO, we used a variety of bone marrow/stromal cell coculture methods. Initially, we found that TPO mainly exerted its effect on the early stage of osteoclastic differentiation in delayed addition experiments. Consequently, the majority of TPO's inhibition of osteoclastic cell formation was due to its effect on bone marrow cells. Finally, we examined whether transforming growth factor-beta (TGF beta) and platelet-derived growth factor (PDGF), major cytokines produced by megakaryocytes, mediate the inhibitory effect of TPO. The addition of either anti-TGF beta or anti-PDGF %antibody% to bone marrow cell culture completely antagonized the effect of TPO on osteoclastogenesis. Furthermore, treatment of bone marrow cells with TGF beta or PDGF mimicked the inhibitory effect of TPO. These data suggest that TPO inhibits osteoclastogenesis through stimulating thrombopoiesis and that TGF beta and PDGF mediate the effect of TPO by impacting on macrophage-lineage cells as osteoclast precursors.

7/3,AB/105 (Item 12 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06139766 Genuine Article#: XX399 Number of References: 44 Title: Cloning and characterization of the mouse vitamin D receptor promoter
Author(s): Jehan F; DeLuca HF (REPRINT)
Corporate Source: UNIV WISCONSIN,COLL AGR & LIFE SCI, DEPT BIOCHEM, 420 HENRY MALL/MADISON//WI/53706 (REPRINT); UNIV WISCONSIN,COLL AGR & LIFE SCI, DEPT BIOCHEM/MADISON//WI/53706
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, %1997%, V94, N19 (SEP 16), P10138-10143 ISSN: 0027-8424 Publication date: 19970916
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418
Language: English Document Type: ARTICLE
Abstract: The gene encoding the mouse vitamin D receptor has been cloned. A new exon 1 has been found that changes the numbering established for the human VDR gene, Exons 2 and 3 in the human VDR gene (coding for the zinc fingers 1 and 2, respectively) are named exons 3 and 4 in the mouse vitamin D receptor. The 1.5-kb 5'-flanking region of the new exon 1 was analyzed and revealed the presence of putative cis-acting elements. Despite the absence of a TATA box, this 5'-flanking region contains several characteristics of a GC-rich promoter including four Sp1 sites present in tandem and two CCAAT boxes. Interestingly, the Sp1 site that is the most proximal to the new exon 1 overlaps a perfect site for Krox-20/24. Krox-20 is a transcription factor involved in brain development, and also in bone remodeling, In luciferase reporter gene expression assays, we showed that sequences from this 5'-flanking region elicit high transactivation activity, Furthermore, in the NIH 3T3 cell line, a 3- to 5-fold increase in response to forskolin treatment (an activator of adenylate cyclase and in turn of protein kinase A pathway) was observed.

7/3,AB/106 (Item 13 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

06039091 Genuine Article#: XR057 Number of References: 33 Title: Thrombopoietin-independent effect of interferon-gamma on the proliferation of human megakaryocyte progenitors
Author(s): Muraoka K; Tsuji K; Yoshida M; Ebihara Y; Yamada K; Sui XW; Tanaka R; Nakahata T (REPRINT)
Corporate Source: UNIV TOKYO,INST MED SCI, DEPT CLIN ONCOL, MINATO KU, 4-6-1 SHIROKANEDAI/TOKYO 108//JAPAN/ (REPRINT); UNIV TOKYO,INST MED SCI, DEPT CLIN ONCOL, MINATO KU/TOKYO 108//JAPAN/
Journal: BRITISH JOURNAL OF HAEMATOLOGY, %1997%, V98, N2 (AUG), P265-273 ISSN: 0007-1048 Publication date: 19970800

Publisher: BLACKWELL SCIENCE LTD, OSNEY MEAD, OXFORD, OXON, ENGLAND OX2 0EL Language: English Document Type: ARTICLE
Abstract: Flow cytometric study revealed that almost all CD34(+) cells in human umbilical cord blood expressed interferon-gamma receptor (IFN-gamma R). To clarify the precise functional roles of IFN-gamma R in human CD34(+) cells, we examined the effect of IFN-gamma alone and in combination with various cytokines on the growth of haemopoietic progenitor cells in CD34(+) cells using a serum-free clonal culture. Surprisingly, IFN-gamma alone supported only megakaryocyte (MK) colonies in a dose-dependent manner with a plateau level at 1000 U/ml of IFN-gamma. IFN-gamma at 1000 U/ml induced 10 +/- 1.2 MK colonies from 1 x 10(3) CD34(+) cells, whereas thrombopoietin (TPO), interleukin (IL)-3, stem cell factor (SCF) or IL-6 alone induced 22 +/- 4.0, 22 +/- 4.2, 4 +/- 0.6 and 0 MK colonies, respectively. The addition of anti-IFN-gamma monoclonal %antibody% (mAb) to the IFN?I culture completely abrogated MK colony formation, whereas the mAb had no effect on TPO-dependent production of MK colonies, In contrast, although anti-TPO polyclonal Ab almost completely blocked TPO-dependent MK colony formation, it failed to inhibit the generation of MK colonies induced by IFN-gamma, suggesting that the observed effect of IFN-gamma on the proliferation of human MK progenitor cells is independent of TPO. The addition of IFN-gamma to culture with TPO or SCF significantly augmented the development of MK colonies, whereas it did not affect IL-3-dependent MK colony formation. Additionally, IFN-gamma induced the increase of DNA content of cultured glycoprotein IIb/IIIa-positive megakaryocytes. These results suggest that IFN-gamma may have regulatory roles in human megakaryocytopoiesis.

7/3,AB/107 (Item 14 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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06008038 Genuine Article#: XN875 Number of References: 51 Title: Cloning and expression pattern of a second
[His(5)Trp(7)Tyr(8)]gonadotropin-releasing hormone (chicken GnRH-II) mRNA in goldfish: Evidence for two distinct genes
Author(s): Lin XW (REPRINT) ; Peter RE
Corporate Source: UNIV ALBERTA,DEPT BIOL SCI/EDMONTON/AB T6G 2E9/CANADA/ (REPRINT)
Journal: GENERAL AND COMPARATIVE ENDOCRINOLOGY, %1997%, V107, N2 (AUG), P 262-272
ISSN: 0016-6480 Publication date: 19970800
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495
Language: English Document Type: ARTICLE
Abstract: Complementary DNAs (cDNAs) encoding [Trp(7)Leu(8)]GnRH (sGnRH) and [His(5)Trp(7)Tyr(8)]GnRH (cGnRH-II) peptides have been isolated from the brain of goldfish (X. W. Lin and R. E. Peter, 1996, Gen. Comp. Endocrinol. 101, 282-296). In the present study we report the isolation of a second cDNA encoding cGnRH-II peptide in the brain of goldfish using reverse transcription (RT) and rapid amplification of cDNA ends. There is an overall 79.7% nucleotide sequence similarity between the two cGnRH-II cDNAs, with 65.3, 91.2, and 76.3% similarity between the 5'-untranslated regions, coding regions, and 3'-untranslated regions, respectively, of the two cGnRH-II cDNAs. Comparison of the two cGnRH-II precursors shows 87.2% amino acid similarity. The presence of two cGnRH-II genes was confirmed by the sequence analysis of the introns between exon II and exon III of the two cGnRH-II genes. Results indicate that the intron of the two cGnRH-II genes shows a high divergence in size and sequence, but contains the same splice junction. Expression of the two cGnRH-II mRNAs was detected by RT-polymerase chain reaction assay and Southern blot analysis in all five grossly dissected brain areas, olfactory bulbs and tracts, telencephalon, hypothalamus, optic tectum-thalamus, and posterior brain. However, there was a difference in apparent intensity of hybridization signal for the two cGnRH-II mRNAs in all brain areas, suggesting a difference of expression levels. sGnRH mRNA was detected in the olfactory bulbs, telencephalon, and hypothalamus, but not in midbrain and posterior brain areas. The present finding of duplicate cDNAs and genes for cGnRH-II in goldfish is in agreement with the recent tetraploidization in this species. (C) 1997 Academic Press.

7/3,AB/108 (Item 15 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05818333 Genuine Article#: WZ706 Number of References: 36 Title: Serum thrombopoietin level is not regulated by transcription but by the total counts of both megakaryocytes and platelets during thrombocytopenia and thrombocytosis
Author(s): Nagata Y; Shozaki Y; Nagahisa H; Nagasawa T; Abe T; Todokoro K (REPRINT)
Corporate Source: INST PHYS & CHEM RES,TSUKUBA LIFE SCI CTR, RIKEN, 3-1 KOYADAI/TSUKUBA/IBARAKI 305/JAPAN/ (REPRINT); INST PHYS & CHEM RES,TSUKUBA LIFE SCI CTR, RIKEN/TSUKUBA/IBARAKI 305/JAPAN/; UNIV TSUKUBA,INST CLIN MED, DIV

HEMATOL/TSUKUBA/IBARAKI 305/JAPAN/ Journal: THROMBOSIS AND HAEMOSTASIS, %1997%, V77, N5 (MAY), P808-814 ISSN: 0340-6245 Publication date: 19970500
Publisher: F K SCHATTAUER VERLAG GMBH, P O BOX 10 45 45, LENZHALDE 3, D-70040 STUTTGART, GERMANY
Language: English Document Type: ARTICLE
Abstract: Thrombopoietin (Tpo) regulates platelet production, but the mechanisms regulating the serum Tpo level and platelet count in circulation have been a subject of debate. Tpo was reported to be expressed mainly in liver and kidney? but we found that Tpo is expressed in all tissues examined: abundantly in liver, kidney, muscle, colon, brain and intestine, and moderately in bone marrow, spleen, lung, stomach, heart, thymus, ovary, and endothelial and leukemic cell lines. The levels of Tpo transcripts in major Tpo producing organs, liver and kidney, and in the platelet production sites bone marrow and spleen, were constant during acute thrombocytopenia induced by anti-platelet monoclonal %antibody% administration in mice, and during thrombocytosis induced by Tpo injection. Furthermore, we noticed that platelet count is not exactly inversely proportional to serum Tpo level. During acute thrombocytopenia, serum Tpo level transiently increased a few hours after %antibody% injection, and returned to the basal level just when matured megakaryocytes accumulated in bone marrow and spleen but the platelet count was still low. Matured megakaryocytes in bone marrow and spleen increased when the serum Tpo level decreased, and decreased when platelet count rebounded. Taken together with other observations, we propose here a modified version of Kuter and Rosenberg's theory, that is, Tpo is constitutively expressed in a variety of organs throughout the body, even in acute thrombocytopenia and thrombocytosis, and that the serum Tpo level is not regulated by Tpo gene expression nor only by platelet counts in circulation, but by the total counts of both megakaryocytes in bone marrow and spleen and of platelets in circulation.

7/3,AB/109 (Item 16 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05815644 Genuine Article#: WZ323 Number of References: 74 Title: Positional cloning of the mouse circadian Clock gene Author(s): King DP; Zhao YL; Sangoram AM; Wilsbacher LD; Tanaka M; Antoch MP; Steeves TDL; Vitaterna MH; Kornhauser JM; Lowrey PL; Turek FW; Takahashi JS (REPRINT)
Corporate Source: NORTHWESTERN UNIV,DEPT NEUROBIOL & PHYSIOL, NATL SCI FDN CTR BIOL TIMING, 2153 SHERIDAN RD/EVANSTON//IL/60208 (REPRINT); NORTHWESTERN UNIV,DEPT NEUROBIOL & PHYSIOL, NATL SCI FDN CTR BIOL TIMING/EVANSTON//IL/60208
Journal: CELL, %1997%, V89, N4 (MAY 16), P641-653 ISSN: 0092-8674 Publication date: 19970516
Publisher: CELL PRESS, 1050 MASSACHUSETTES AVE, CIRCULATION DEPT, CAMBRIDGE, MA 02138
Language: English Document Type: ARTICLE
Abstract: We used positional cloning to identify the circadian Clock gene in mice. Clock is a large transcription unit with 24 exons spanning similar to 100,000 bp of DNA from which transcript classes of 7.5 and similar to 10 kb arise. Clock encodes a novel member of the bHLH-PAS family of transcription factors. In the Clock mutant allele, an A-->T nucleotide transversion in a splice donor site causes exon skipping and deletion of 51 amino acids in the CLOCK protein. Clock is a unique gene with known circadian function and with features predicting DNA binding, protein dimerization, and activation domains. CLOCK represents the second example of a PAS domain-containing clock protein (besides Drosophila PERIOD), which suggests that this motif may define an evolutionarily conserved feature of the circadian clock mechanism.

7/3,AB/110 (Item 17 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05792935 Genuine Article#: WY123 Number of References: 33 Title: Altered mRNA expression due to insertion or substitution of thymine at position +3 of two splice-donor sites in the androgen receptor gene Author(s): Trifiro MA (REPRINT) ; Lumbroso R; Beitel LK; Vasiliou DM; Bouchard J; Deal C; VanVliet G; Pinsky L
Corporate Source: SIR MORTIMER B DAVIS JEWISH HOSP,LADY DAVIS INST MED RES, DEPT MED, 3755 COTE ST CATHERINE RD/MONTREAL/PQ H3T E21/CANADA/ (REPRINT); SIR MORTIMER B DAVIS JEWISH HOSP,LADY DAVIS INST MED RES, DEPT BIOL/MONTREAL/PQ H3T E21/CANADA/; SIR MORTIMER B DAVIS JEWISH HOSP,LADY DAVIS INST MED RES, DEPT HUMAN GENET/MONTREAL/PQ H3T E21/CANADA/; MCGILL UNIV,DEPT PEDIAT/MONTREAL/PQ H3A 2T5/CANADA/; HOP ST JUSTINE,/MONTREAL/PQ H3T 1C5/CANADA/
Journal: EUROPEAN JOURNAL OF HUMAN GENETICS, %1997%, V5, N1 (JAN-FEB), P 50-58
ISSN: 1018-4813 Publication date: 19970100
Publisher: KARGER, ALLSCHWILERSTRASSE 10, CH-4009 BASEL, SWITZERLAND Language: English
Document Type: ARTICLE
Abstract: We have discovered two types of 5' intronic gene mutation that impair androgen receptor (AR) mRNA expression severely, and cause complete

androgen insensitivity, Labium majus skin fibroblasts (LMSF) hemizygous for each mutation had negligible specific androgen binding, and did not react to an %antibody% against an N-terminal peptide of the AR. Both mutations were detected by direct sequencing of exons PCR-amplified with flanking primers, One mutation is an adenine to thymine transversion at position +3 of the intron 6 splice-donor site, Using LMSF mRNA, RT-PCR of a portion of the AR androgen-binding domain yielded a small amount of a 302-bp mutant fragment instead of a 433-bp wild-type product, Sequencing established that exon 5 was followed, out of frame, by exon 7: exon 6 was skipped. The other mutation is a thymine insertion at the +3 position of the intron 1 donor-splice site, RT-PCR and sequencing revealed a small amount of normal-size mRNA with normal exon 1-exon 2 splicing, Quantitative RT-PCR on mutant LMSF showed AR mRNA levels were well below 10% of normal; hence, most of the aberrant AR mRNA resulting from each mutation is probably unstable, The misbehavior caused by these two mutations indicates that in the AR the splice-donor site +3 adenine is critical; indeed, 57% of eukaryotic introns have adenine in the +3 position, while only 2% have thymine.

7/3,AB/111 (Item 18 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05787934 Genuine Article#: WX516 Number of References: 337 Title: Interleukin-6: Structure-function relationships
Author(s): Simpson RJ (REPRINT) ; Hammacher A; Smith DK; Matthews JM; Ward LD
Corporate Source: ROYAL MELBOURNE HOSP,LUDWIG INST CANC RES, POB 2008/MELBOURNE/VIC 3050/AUSTRALIA/ (REPRINT); WALTER & ELIZA HALL INST MED RES,/PARKVILLE/VIC 3050/AUSTRALIA/; LUDWIG INST CANC RES,MELBOURNE TUMOUR BIOL BRANCH, JOINT PROT STRUCT LAB/PARKVILLE/VIC 3050/AUSTRALIA/ ; COOPERAT RES CTR CELLULAR GROWTH FACTORS,/PARKVILLE/VIC 3050/AUSTRALIA/
Journal: PROTEIN SCIENCE, %1997%, V6, N5 (MAY), P929-955
ISSN: 0961-8368 Publication date: 19970500
Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211
Language: English Document Type: REVIEW
Abstract: Interleukin-6 (IL-6) is a multifunctional cytokine that plays a central role in host defense due to its wide range of immune and hematopoietic activities and its potent ability to induce the acute phase response. Overexpression of IL-6 has been implicated in the pathology of a number of diseases including multiple myeloma, rheumatoid arthritis, Castleman's disease, psoriasis, and post-menopausal osteoporosis. Hence, selective antagonists of IL-6 action may offer therapeutic benefits. IL-6 is a member of the family of cytokines that includes interleukin-11, leukemia inhibitory factor, oncostatin M, cardiotrophin-1 and ciliary neurotrophic factor. Like the other members of this family, IL-6 induces growth or differentiation via a receptor-system that involves a specific receptor and the use of a shared signaling subunit, gp130. Identification of the regions of IL-6 that are involved in the interactions with the IL-6 receptor and gp130 is an important first step in the rational manipulation of the effects of this cytokine for therapeutic benefit. In this review, we focus on the sites on IL-6 which interact with its low-affinity specific receptor, the IL-6 receptor, and the high-affinity converter gp130. A tentative model for the IL-6 hexameric receptor ligand complex is presented and discussed with respect to the mechanism of action of the other members of the IL-6 family of cytokines.

7/3,AB/112 (Item 19 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05775645 Genuine Article#: WX362 Number of References: 32 Title: Thrombin cleaves recombinant human thrombopoietin: One of the proteolytic events that generates truncated forms of thrombopoietin
Author(s): Kato T (REPRINT) ; Oda A; Inagaki Y; Ohashi H; Matsumoto A; Ozaki K; Miyakawa Y; Watarai H; Fuju K; Kokubo A; Kadoya T; Ikeda Y; Miyazaki H
Corporate Source: KIRIN BREWERY CO LTD,PHARMACEUT RES LAB, 3 MIYAHARA CHO/TAKASAKI/GUMMA 37012/JAPAN/ (REPRINT); KEIO UNIV,DEPT INTERNAL MED, DIV HEMATOL, SHINJUKU KU/TOKYO 160//JAPAN/
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, %1997%, V94, N9 (APR 29), P4669-4674
ISSN: 0027-8424 Publication date: 19970429
Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC 20418
Language: English Document Type: ARTICLE
Abstract: A heterogeneity in the molecular weight (M-r) of thrombopoietin (TPO) has been reported, We found several thrombin cleavage sites in human, rat, murine, and canine TPOs, and also found that human TPO undergoes selective proteolysis by thrombin, Recombinant human TPO (rhTPO) was incubated with human platelets in the presence of calcium ions to allow the generation of thrombin, and was cleaved into low M-r peptide fragments, The cleavage was completely inhibited by hirudin, indicating that the proteolysis was mediated by

thrombin. In a platelet-free system, analyses of thrombin cleavage by immunoblotting using anti-human TPO peptide %antibodies% revealed that the four major thrombin-cleaved peptide fragments were selectively generated depending on the digestion time, The amino acid sequences of the thrombin-polypeptides were further analyzed, and two major thrombin cleavage sites were determined, One of them was at AR(191)-T-192 in the C-terminal domain of TPO, and thrombin cleaved first at this site, The other site at GR(117)-T-118 in the N-terminal domain was subsequently cleaved by prolonged thrombin digestion, As a result, the biological activity of TPO was modulated, The generation of truncated forms of TPO by thrombin may be a notable event in view of the platelet-related metabolism of TPO.

7/3,AB/113 (Item 20 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05774960 Genuine Article#: WX253 Number of References: 43 Title: Platelet procoagulant activity during peripheral blood stem cell harvest
Author(s): Katsura K; Nomura S (REPRINT) ; Xie GL; Ohtani T; Ishida T; Kagawa H; Kitada C; Yamanaka Y; Kitajima H; Fukuhara S Corporate Source: KANSAI MED UNIV,DEPT INTERNAL MED 1, 10-15 FUMNIZONO CHO/MORIGUCHI/OSAKA 570/JAPAN/ (REPRINT); KANSAI MED UNIV,DEPT INTERNAL MED 1/MORIGUCHI/OSAKA 570/JAPAN/; KANSAI MED UNIV,DEPT BLOOD TRANSFUS/OSAKA//JAPAN/
Journal: CLINICAL AND APPLIED THROMBOSIS-HEMOSTASIS, %1997%, V3, N2 (APR) , P124-128
ISSN: 1076-0296 Publication date: 19970400
Publisher: LIPPINCOTT-RAVEN PUBL, 227 EAST WASHINGTON SQ, PHILADELPHIA, PA 19106
Language: English Document Type: ARTICLE
Abstract: We used flow cytometry to measure platelet-derived microparticle levels in plasma obtained from 16 patients during peripheral blood stem cell harvest (PBSC) and in platelet concentrates prepared by apheresis from 10 normal controls. We also studied the binding of an anti-P-selectin %antibody% and annexin-V to platelets. When all 60 harvests were assessed, we noted a significant difference in microparticle levels between patients with a platelet count >10 X 10(4)/mu l and those with a platelet count <10 x 10(4)/mu l (12.3 +/- 4.4 vs. 7.5 +/- 3.9%). In both the first and total harvests, the percentage of platelets and microparticles positive for anti-P-selectin and annexin-V were significantly higher than the normal control levels. These results suggest that patients undergoing mobilization by granulocyte colony-stimulating factor (G-CSF) who have a platelet count >10 x 10(4)/mu l are at risk of increased procoagulant activity after retransfusion following PBSC harvest.

7/3,AB/114 (Item 21 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05740601 Genuine Article#: WU761 Number of References: 39 Title: Endothelial-selectin ligands sialyl Lewis(x) and sialyl Lewis(a) are differentiation antigens immunogenic in human melanoma Author(s): Ravindranath MH (REPRINT) ; Amiri AA; Bauer PM; Kelley MC; Essner R; Morton DL
Corporate Source: ST JOHNS HOSP,JOHN WAYNE CANC INST, GLYCOLIPID IMMUNOTHERAPY LAB/SANTA MONICA//CA/90404 (REPRINT); ST JOHNS HOSP,JOHN WAYNE CANC INST, SONYA VALLEY GHIDOSSI VACCINE LAB/SANTA MONICA//CA/90404; ST JOHNS HOSP,JOHN WAYNE CANC INST, ROY E COATS RES LABS/SANTA MONICA//CA/90404
Journal: CANCER, %1997%, V79, N9 (MAY 1), P1686-1697
ISSN: 0008-543X Publication date: 19970501
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC 605 THIRD AVE, NEW YORK, NY 10158-0012
Language: English Document Type: ARTICLE
Abstract: BACKGROUND. Sialyl Lewis(x) (sLe(x)) and sialyl Lewis(a) (sLe(n)), the endothelial-selectin ligands involved in extravasation of neutrophils and carcinomas, have been identified in human melanoma. This study explored the following issue: If these ligands are immunogenic tumor-differentiation antigens, they would be potential targets for immunotherapy because of their putative roles in extravasation and metastasis.

METHODS. Using a cell-suspension enzyme-linked immunosorbent assay (ELISA), the expression of sLe(x) and sLe(a) on the surface of normal melanocytes, melanoma cells from biopsies, and cell lines (M10-v, M24, and M101) constituting melanoma cell vaccine (MCV) were quantitated. Melanoma patients were immunized with the MCV expressing these antigens. Sera of normal individuals, sera of patients, and sera that adsorbed to sLe(x) and sLe(a) were titrated for anti-sie %antibodies% by ELISA to verify the immunogenicity of the ligands.

RESULTS. The normal melanocytes did not express sLe(x) and poorly expressed sLe(a). Melanoma cells from tumor biopsies and MCV lines expressed both sLe(x) and sLe(a). Sialyl Le(x) was associated with glycoprotein(s) in M10-v, and sLe(a) occurred as a glycolipid moiety in M24. MCV recipients developed high titers for immunoglobulin (Ig)M but not IgG to both ligands. IgM titers to these ligands were low in normal

subjects. In some of the preimmune sera of patients, the titers were threefold above normal. Six of 13 MCV recipients developed at least a twofold increase in anti-sie titers above preimmune level after the second or third immunization. Adsorption studies suggested that both ligands were immunogenic.

CONCLUSIONS. The melanoma-associated sLe(x) and sLe(n) are immunogenic neoplasm-differentiation antigens and are therefore potential targets for passive and active specific immunotherapy in the treatment of melanoma. (C) 1997 American Cancer Society.

7/3,AB/115 (Item 22 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05682078 Genuine Article#: WQ067 Number of References: 41 Title: The leptin receptor activates janus kinase 2 and signals for proliferation in a factor-dependent cell line
Author(s): Ghilardi N; Skoda RC (REPRINT)
Corporate Source: UNIV BASEL,BIOZENTRUM, KLINGELBERGSTR 70/CH-4056 BASEL//SWITZERLAND/ (REPRINT); UNIV BASEL,BIOZENTRUM/CH-4056 BASEL//SWITZERLAND/
Journal: MOLECULAR ENDOCRINOLOGY, %1997%, V11, N4 (APR), P393-399 ISSN: 0888-8809 Publication date: 19970400
Publisher: ENDOCRINE SOC, 4350 EAST WEST HIGHWAY SUITE 500, BETHESDA, MD 20814-4110
Language: English Document Type: ARTICLE
Abstract: The antiobesity effects of leptin are mediated by the obese receptor (OB-R), a member of the cytokine receptor superfamily. Several isoforms of OB-R that differ in the length of the cytoplasmic domain have been described. An isoform with a long cytoplasmic domain of 302 amino acids, termed OB-Rb, contains the conserved box 1 and box 2 motifs and is likely to be responsible for leptin-induced signaling. A point mutation in the OB-R gene of diabetes (db) mice generates a new splice donor that interferes with the correct splicing of the OB-Rb mRNA and is predicted to cause absence of the OB-Rb protein in db/db mice. Here we examined the signaling potential of the long isoform, OB-Rb, and of a short isoform, OB-Ra, in BaF3 cells, a factor-dependent hematopoietic cell line. The long isoform was able to generate a proliferative signal and upon leptin binding, activated janus kinase 2 (Jak2). Consistently, %antibodies% directed against the extracellular domain of OB-R coprecipitated Jak2. The short isoform, OB-Ra, was inactive in both proliferation and Jak activation. These results provide further support for the long isoform, OB-Rb, being the principal mediator of the effects of leptin and help to explain why db/db mice are resistant to leptin, despite the presence of the short OB-R isoforms.

7/3,AB/116 (Item 23 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05629674 Genuine Article#: WL989 Number of References: 41 Title: Molecular characterisation of an expressed sequence tag locus of Toxoplasma gondii encoding the micronemal protein MIC2 Author(s): Wan KL; Carruthers VB; Sibley LD; Ajioka JW (REPRINT)
Corporate Source: UNIV CAMBRIDGE,DEPT PATHOL, TENNIS COURT RD/CAMBRIDGE CB2 1QP//ENGLAND/ (REPRINT); UNIV CAMBRIDGE,DEPT PATHOL/CAMBRIDGE CB2 1QP//ENGLAND/; WASHINGTON UNIV,SCH MED, DEPT MOL MICROBIOL/ST LOUIS//MO/
Journal: MOLECULAR AND BIOCHEMICAL PARASITOLOGY, %1997%, V84, N2 (FEB), P 203-214
ISSN: 0166-6851 Publication date: 19970200
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS Language: English
Document Type: ARTICLE
Abstract: The expressed sequence tag (EST) dataset of Toxoplasma gondii provides a wealth of information towards gene discovery. The complete cDNA and genomic sequence of EST tgc050 locus shows that it contains five copies of the conserved thrombospondin (TSP)-like motif present in a number of molecules with adhesive properties. A conserved region implicated with the adhesive characteristic of another group of proteins including several integrins, is also present in this molecule. The protein encoded by this sequence (rc50) is strongly recognised by monoclonal %antibodies% to MIC2. Affinity purified anti-rc50 antisera specifically reacted with a single protein of identical molecular mass as MIC2 and exclusively labeled the micronemes of T. gondii by cryo-immunoelectron microscopy. These results demonstrate that c50 encodes for MIC2, a previously characterised microneme protein of T. gondii. The extensive sequence similarity across multiple protein domains provides evidence that the protein encoded by this locus is the homologue to the Etp100 microneme protein of Eimeria tenella. (C) 1997 Elsevier Science B.V.

7/3,AB/117 (Item 24 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05554510 Genuine Article#: WG073 Number of References: 60 Title: Thrombopoietin enhances the alpha(IIb)beta(3)-dependent adhesion of

megakaryocytic cells to fibrinogen or fibronectin through PI 3 kinase Author(s): Zauli G (REPRINT) ; Bassini A; Vitale M; Gibellini D; Celeghini C; Caramelli E; Pierpaoli S; Guidotti L; Capitani S
Corporate Source: UNIV FERRARA,INST HUMAN ANAT, VIA FOSSATO MORTARA 66/I-44100 FERRARA//ITALY/ (REPRINT); UNIV BOLOGNA,INST HISTOL & GEN EMBRYOL/BOLOGNA//ITALY/; UNIV BRESCIA,SECT HUMAN ANAT, DEPT BIOMED SCI & TECHNOL/BRESCIA//ITALY/; INST CODIVILLA PUTTI,CNR, INST NORMAL & PATHOL CYTOMORPHOL/BOLOGNA//ITALY/; UNIV BOLOGNA,INST MICROBIOL/BOLOGNA//ITALY/
Journal: BLOOD, %1997%, V89, N3 (FEB 1), P883-895
ISSN: 0006-4971 Publication date: 19970201
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399
Language: English Document Type: ARTICLE
Abstract: The effect of thrombopoietin (TPO) on the functional activity of surface alpha(IIb)beta(3) (GPIIbIIIa) was investigated in both primary human megakaryocytic cells, derived from peripheral blood CD34(+) cells, and HEL hematopoietic cell line. TPO (100 ng/mL) induced a sixfold to ninefold enhancementof adhesion of both primary megakaryocytic and HEL cells to plates coated with either fibrinogen or fibronectin and a parallel increase of immunoreactivity to the PAC1 monoclonal %antibody% (MoAb) and fluorescein isothiocyanate-fibrinogen, both of which recognize an activated state of alpha(IIb)beta(3). The enhanced adhesion to fibrinogen or fibronectin was mediated by the Arg-Gly-Asp (RGD) recognition sequence of alpha(IIb)beta(3) as if was abolished by pretreatment of cells with saturating concentrations of RGDS peptide. A MoAb specific for the alpha(IIb) subunit of alpha(IIb)beta(3) also inhibited cell attachment to fibrinogen or fibronectin, while MoAb to anti-alpha(v) beta(3) or anti alpha 5 integrins were completely ineffective, clearly indicating that alpha(IIb)beta(3) participates in this association. A role for PI 3 kinase (PI 3-K) in the TPO-mediated increase in alpha(IIb)beta(3) function in megakaryocytic cells was suggested by the ability of the PI 3-K inhibitor wortmannin (100 nmol/L) and antisense oligonucleotides directed against the p85 regulatory subunit of PI 3-K to completely block the TPO-induced increase in alpha(IIb)beta(3) integrin activity upon TPO stimulation. The modulation of adhesiveness to extracellular matrix proteins containing the RGD motif mediated by TPO likely plays a physiologic role in megakaryocytopoiesis, as pretreatment of CD34(+) cells with RGDS or anti-alpha(IIb) MoAb significantly reduced the number of megakaryocytic colonies obtainedin a fibrinclot semisolid assay. (C) 1997 by The American Society of Hematology.

7/3,AB/118 (Item 25 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05554504 Genuine Article#: WG073 Number of References: 74 Title: A single injection of pegylated murine megakaryocyte growth and development factor (MGDF) into mice is sufficient to produce a profound stimulation of megakaryocyte frequency, size, and ploidization Author(s): Arnold JT; Daw NC; Stenberg PE; Jayawardene D; Srivastava DK; Jackson CW (REPRINT)
Corporate Source: ST JUDE CHILDRENS HOSP,DIV EXPT HEMATOL, 332 N LAUDERDALE ST/MEMPHIS//TN/38105 (REPRINT); ST JUDE CHILDRENS HOSP,DIV EXPT HEMATOL/MEMPHIS//TN/38105; ST JUDE CHILDRENS HOSP,DEPT BIOSTAT/MEMPHIS//TN/38105; OREGON HLTH SCI UNIV,DEPT PATHOL/PORTLAND//OR/97201
Journal: BLOOD, %1997%, V89, N3 (FEB 1), P823-833
ISSN: 0006-4971 Publication date: 19970201
Publisher: W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399
Language: English Document Type: ARTICLE
Abstract: Despite numerous studies investigating the action of c-%mpl% ligand, no reports have defined the in vivo changes in megakaryocytopoiesis in response toa single injection of this cytokine. Here we compare the kinetics of the megakaryocytopoietic response in C57B1/6J mice administered 25 mu g/kg or 250 mu g/kg of pegylated (PEG) murine megakaryocyte growth and development factor (MGDF) as a single intravenous injection. Megakaryocytes of mice treated with MGDF had normal ultrastructure, showing a typical distribution of the demarcation membrane system, alpha-granules, and other cytoplasmic organelles. Megakaryocyte ploidy, size, and frequency were markedly increased with both MGDF doses. Megakaryocyte ploidy was maximally increased from a modal value of 16N to 64N on day 3, with both doses of MGDF. Similarly, a comparable increase in megakaryocyte size occurred in the two MGDF groups. Increased megakaryocyte size was coupled to the increase in megakaryocyte ploidy, and no evidence for independent regulation of megakar/ocyte size within individual ploidy classes was apparent. In contrast to megakaryocyte ploidyand size, the increase in megakaryocyte frequency was markedly different with the two doses of MGDF. The proportion of 2N and 4N cells was increased from a baseline of 0.035% to 0.430%. by day 4 in mice treated with the higher dose of MGDF, but only to 0.175% in mice administered 25 mu

g/kg of MGDF. The marked increase in the pool of these immature megakaryocytes translated to a sustained elevation in the frequency of polyploid megakaryocytes (8N cells and greater). In contrast to the sustained increase in the frequency of polyploid cells, the level of polyploidization was downregulated on days 6 to 10, but normalized by day 14. We conclude that a single injection of MGDF is able to expand the megakaryocytic pool in a dose-dependent manner, which, with subsequent maturation, should lead to an increased rate of platelet production. (C) 1997 by The American Society of Hematology.

7/3,AB/119 (Item 26 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05552943 Genuine Article#: WG065 Number of References: 23 Title: Molecular analysis of mutated thyroid peroxidase detected in patients withtotal iodide organification defects
Author(s): Bikker H (REPRINT) ; Baas F; DeVijlder JJM
Corporate Source: UNIV AMSTERDAM,EMMA CHILDRENS HOSP AMC, ACAD MED CTR, H2-255, POB 22700/NL-1100 DE AMSTERDAM//NETHERLANDS/ (REPRINT); DEPT NEUROL,/AMSTERDAM//NETHERLANDS/
Journal: JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, %1997%, V82, N2 (FEB), P649-653
ISSN: 0021-972X Publication date: 19970200
Publisher: ENDOCRINE SOC, 4350 EAST WEST HIGHWAY SUITE 500, BETHESDA, MD 20814-4110
Language: English Document Type: ARTICLE
Abstract: Wild-type and mutant thyroid peroxidase (TPO) was expressed in a Semliki Forest Virus (SFV)-based transient expression system in Chinese hamster ovary-K1 cells. Twenty four hours after transfection, proteins immunoreactive with TPO %antibodies% could be detected on a Western blot. Peroxidase activitywas assayed using both the guaiacol and the I-3(-) assay. Addition of hematin was necessary to obtain enzymatic active TPO. Thyroid peroxidase complementary DNA constructs containing mutations originally found in patients with hereditary congenital hypothyroidism caused by total iodide organification defects were analysed using these techniques. In all cases TPO was expressed as shown by Western blotting and immunostaining. Enzymatic activity (measured by guaiacol and iodide oxidation assay) was below the detection level in four out of five mutants. The only mutant yielding TPO with enzymaticactivity was G 1858 A (Gly 590 Ser). However, the mutation could affect splicing of TPO messenger RNA, leading to inactive TPO, because it is locatedat the exon 10/intron 10 border.

7/3,AB/120 (Item 27 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05528671 Genuine Article#: WE138 Number of References: 68 Title: Differentiation of haematopoietic cells: Role of cytokines and expression of membrane markers.
Author(s): Bidri M; Arock M (REPRINT)
Corporate Source: FAC MED & PHARM BESANCON,HEMATOL LAB, PL ST JACQUES/F-25030 BESANCON//FRANCE/ (REPRINT); FAC PHARM,LAB HEMATOL CELLULAIRE & MOL/F-75006 PARIS//FRANCE/
Journal: REVUE FRANCAISE D ALLERGOLOGIE ET D IMMUNOLOGIE CLINIQUE, %1996% , V36, N8 (DEC), P859-878
ISSN: 0335-7457 Publication date: 19961200
Publisher: EXPANSION SCI FRANCAISE, 31 BLVD LATOUR MAUBOURG, 75007 PARIS, FRANCE
Language: French Document Type: ARTICLE
Abstract: Haematopoiesis represents all of the mechanisms ensuring continuous replacement of mature blood cells with a very limited or no capacity for proliferation and a limited life span. This regulated and continuous production is ensured by a special cell pool, termed stem cells, capable of multiplication without differentiation (self-renewal) and proliferation with differentiation into one or other of the Various specialized haematopoietic cells: erythrocytes, neutrophil, eosinophil and basophil polymorphonuclear cells, platelets, monocytes/macrophages, T and B lymphocytes. In addition to the bonemarrow compartment corresponding to the most primitive stem cells, the bone marrow also contains two other compartments of more differentiated cells:the progenitor and maturation compartments. Stem cells, which represent anextremely low fraction of the total bone marrow population, are usually quiescent and are entered into cycle under the action of certain soluble glycoprotein factors, cytokines, ensuring their survival, proliferation and differentiation (<< positive >> regulation), but also their interactions with certain cells of the microenvironment and finally the << negative >> regulation activity of haematopoiesis exerted by certain biological molecules. All of the known data concerning the filiation of the various haematopoietic cells belonging to the three compartments defined above has been considerably enriched over recent years with the demonstration of various membrane markers on the surface of these different cells, allowing very precise dissection of the hierarchy of these compartments by means of specific %antibodies%.

7/3,AB/121 (Item 28 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05526436 Genuine Article#: WE245 Number of References: 32 Title: Lecithinization of IL-6 enhances its thrombopoietic activity in mice Author(s): Igarashi R (REPRINT) ; Tsutsumi Y; Fujii H; Tsunoda S; Ochiai A; Takenaga M; Morizawa Y; Mayumi T; Mizushima Y
Corporate Source: ST MARIANNA UNIV,INST MED SCI, DIV DRUG DELIVERY SYST, MIYAMAE KU, 2-16-1 SUGAO/KAWASAKI/KANAGAWA 216/JAPAN/ (REPRINT); ASAHI GLASS CO LTD,RES CTR, KANAGAWA KU/YOKOHAMA/KANAGAWA 221/JAPAN/; OSAKA UNIV,FAC PHARMACEUT SCI/SUITA/OSAKA 565/JAPAN/
Journal: JOURNAL OF PHARMACY AND PHARMACOLOGY, %1997%, V49, N1 (JAN), P 113-118
ISSN: 0022-3573 Publication date: 19970100
Publisher: ROYAL PHARMACEUTICAL SOC GREAT BRITAIN, 1 LAMBETH HIGH ST, LONDON, ENGLAND SE1 7JN
Language: English Document Type: ARTICLE
Abstract: This study was conducted to assess the merit of lecithinization of recombinant human interleukin-6 (IL-6) as a drug delivery system.

IL-6 was lecithinized by covalently binding it with a phosphatidylcholine (lecithin, PC) derivative. The in-vivo thrombopoietic potency of lecithinized IL-6 (PC-IL-6) was greater than that of native IL-6 when administered subcutaneously, although the in-vitro bioactivity of PC-IL-6 was markedly reduced by lecithinization. When PC-IL-6 and native IL-6 were given in doses that produced the same level of thrombopoietic activity, the former stimulated less production of IgG(1), a marker of the adverse effects of IL-6, thandid the latter. Furthermore, PC-IL-6 persisted in the blood longer than native IL-6. Based on the above, PC-IL-6 appears to be useful as a drug delivery system and may also be useful in the treatment of drug-induced thrombocytopenia.

7/3,AB/122 (Item 29 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05466359 Genuine Article#: WA836 Number of References: 70 Title: PREVENTION OF THROMBOCYTOPENIA AND NEUTROPENIA IN A NONHUMAN PRIMATE MODEL OF MARROW SUPPRESSIVE CHEMOTHERAPY BY COMBINING PEGYLATED RECOMBINANT HUMAN MEGAKARYOCYTE GROWTH AND DEVELOPMENT FACTOR AND RECOMBINANT HUMAN GRANULOCYTE-COLONY-STIMULATING FACTOR
Author(s): HARKER LA; MARZEC UM; KELLY AB; CHEUNG E; TOMER A; NICHOL JL; HANSON SR; STEAD RB
Corporate Source: EMORY UNIV,SCH MED,YERKES REG PRIMATE RES CTR,DIV HEMATOL & ONCOL,PO DRAWER AR/ATLANTA//GA/30322; AMGEN INC/THOUSAND OAKS//CA/91320
Journal: BLOOD, %1997%, V89, N1 (JAN 1), P155-165
ISSN: 0006-4971
Language: ENGLISH Document Type: ARTICLE
Abstract: This report examines the effects on hematopoietic regeneration of pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) (2.5 mu g/ kg/d) alone and in combination with recombinant human granulocyte colony stimulating factor (rHu-GCSF) (10 mu g/ kg/d) for 21. days in rhesus macaques receiving intense marrow suppression produced by single bolus injections of hepsulfarn (1.5 g/m(2)). In six hepsulfam-only control animals thrombocytopenia (platelet count <100 x 10(9)/L) was observed between days 12 and 25 (nadir 39 +/- 20 x 10(9)/L on day 17), and neutropenia (absolute neutrophil count <1 x 10(9)/L) occurred between days 8 and 30 (nadir 0.167 +/- 0.120 x 10(9)/L on day 15). PEG-rHuMGDF (2.5 mu g/kg/d) injected subcutaneously into four animals from day 1 to day 22 following hepsulfam administration produced trough serum concentrations of 1.9 +/- 0.2 ng/mL and increased the platelet count twofold over basal prechemotherapy levels (856 +/- 594 x 10(9)/L v baseline of 416 +/- 88 x 10(9)/L; P =.01). PEG-rHuMGDF alone also shortened the period of posthepsulfam neutropenia from 22 days to 12 days (P =.01), although the neutropenic nadir was not significantly altered (neutrophil count 0.224 +/- 0.112 x 10(9)/L v 0.167 +/- 0.120 x 10(9)/L; P > .3). rHu-GCSF (10 mu g/kg/d) injected subcutaneously into four animals from day 1 to day 22 following hepsulfam administration produced trough serum concentrations of 1.4 +/- 1.1 ng/mL, and reduced the time for the postchemotherapy neutrophil count to attain 1 x 10(9)/L from 22 days to 4 days (P =.005). The postchemotherapy neutropenic nadir was 0.554 +/- 0.490 x 10(9)-neutrophils/L (P =.3 v hepsulfam-only control of 0.167 +/- 0.120 x 10(9)/L). However, thrombocytopenia of <100 x 10(9) platelets/L was not shortened (persisted from day 12 to day 25), or less severe (nadir of 56 +/- 32 x 10(9) platelets/L on day 14; P =.7 compared with untreated hepsulfam animals). The concurrent administration of rHu-GCSF (10 mu g/kg/d) and PEG-rHuMGDF (2.5 mu g/kg/d) in four animals resulted in postchemotherapy peripheral platelet counts of 127 +/- 85 x 10(9)/L (P =.03 compared with 39 +/- 20 x 10(9)/L for untreated hepsulfam alone, and P=.02 compared with 856 +/- 594 x 10(9)/L for PEG-rHuMGDF alone), and shortened the period of neutropenia <1 x 10(9)/L from 22 days to 4 days (P =.8 compared with rHu-GCSF alone). Increasing

PEG-rHuMGDF to 10 mu g/kg/d and maintaining the 21-day schedule of coadministration with rHu-GCSF (10 mu g/kg/d) in another four animals produced postchemotherapy platelet counts of 509 +/- 459 x 10(9)/L (P < 10(-4) compared with untreated hepsulfam alone, and P =.04 compared with 2.5 mu g/kg/d PEG-rHuMGDF alone), and 4 days of neutropenia. Coadministration of rHu-GCSF and PEG-rHuMGDF did not significantly alter the pharmacokinetics of either agent. The administration of PEG-rHuMGDF (2.5 mu g/kg/d) from day 1 through day 22 and rHu-GCSF (10 mu g/kg/d) from day 8 through day 22 in six animals produced peak postchemotherapy platelet counts of 747 +/- 317 x 10(9)/L (P < 10(-4) compared with untreated hepsulfam alone, and P =.7 compared with PEG-rHuMGDF alone), and maintained the neutrophil count > 3.5 x 10(9)/L (P =.008 v rHu-GCSF therapy alone). Thus, both thrombocytopenia and neutropenia are eliminated by initiating daily PEG-rHuMGDF therapy on day 1 and subsequently adding daily rHu-GCSF after 1 week in the rhesus model of hepsulfam marrow suppression.

This improvement in platelet and neutrophil responses by delaying the addition of rHu-GCSF to PEG-rHuMGDF therapy demonstrates the importance of optimizing the dose and schedule of cytokine combinations after severe myelosuppressive chemotherapy. (C) 1997 by The American Society of Hematology.

7/3,AB/123 (Item 30 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05424245 Genuine Article#: VX843 Number of References: 59 Title: V(H)4-34(V(H)4.21) GENE-EXPRESSION IN THE CHRONIC ARTHRITIDES OF CHILDHOOD - STUDIES OF ASSOCIATIONS WITH ANTI-LIPID A %ANTIBODIES%, HLA ANTIGENS, AND CLINICAL-FEATURES
Author(s): MILLER JJ; BIEBER MM; LEVINSON JE; ZHU SL; TSOU E; TENG NNH Corporate Source: PACKARD CHILDRENS HOSP,725 WELCH RD/PALO ALTO//CA/94304; STANFORD UNIV,SCH MED,DEPT PEDIAT/STANFORD//CA/94305; STANFORD UNIV,SCH MED,DEPT GYNECOL & OBSTET/STANFORD//CA/94305; CHILDRENS HOSP & MED CTR,DIV RHEUMATOL/CINCINNATI//OH/00000
Journal: JOURNAL OF RHEUMATOLOGY, %1996%, V23, N12 (DEC), P2132-2139 ISSN: 0315-162X
Language: ENGLISH Document Type: ARTICLE
Abstract: Objective. To determine if the germ line gene V(H)4-34 (V(H)4.21) encodes the antimonophosphoryl lipid A (%MPL%) polyspecific %antibodies% found in oligoarticular arthritis of childhood,

Methods. Sera from a range of rheumatic diseases of childhood were assayed for V(H)4-34 derived %antibodies% by ELISA using the antiidiotype monoclonal %antibody% 9G4. Results were compared to assays for anti-%MPL% %antibodies%, C4d, and Bb, and for HLA type, joint count? and sedimentation rate.

Results, V(H)4-34 derived %antibodies% were elevated in all diseases studied except rheumatoid factor positive polyarticular disease, in oligoarticular arthritis, V(H)4-34 gene expression correlated with C4d concentration, and V(H)4-34 encoded globulins were more concentrated in synovial fluid than in blood, No association was found with HLA type. An association between V(H)4-34 expression and IgG anti-%MPL% was found in sera from patients from Cincinnati but not from Stanford. No other evidence sup ported a direct association between V(H)4-34 derived and anti-%MPL% %antibodies% in these children,

Conclusion, The expression of V(H)3-34 is increased in several rheumatic diseases of childhood, but, as in adults, not in rheumatoid arthritis. V(H)4-34 expression is not associated with HLA type. The polyspecific autoantibody nature of some V(H)4-34 derived %antibodies% may explain the wide range of the unusual %antibodies% found in oligoarticular arthritis.

7/3,AB/124 (Item 31 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05387947 Genuine Article#: VV147 Number of References: 43 Title: IN-VIVO EFFECTS OF PEGYLATED RECOMBINANT HUMAN MEGAKARYOCYTE GROWTH AND DEVELOPMENT FACTOR ON HEMATOPOIESIS IN NORMAL MICE Author(s): KABAYA K; AKAHORI H; SHIBUYA K; NITTA Y; IDA M; KUSAKA M; KATO T ; MIYAZAKI H
Corporate Source: KIRIN BREWERY CO LTD,PHARMACEUT RES LAB,3 MIYAHARA CHO/TAKASAKI/GUMMA 37012/JAPAN/; KIRIN BREWERY CO LTD,PHARMACEUT RES LAB/TAKASAKI/GUMMA 37012/JAPAN/
Journal: STEM CELLS, %1996%, V14, N6 (NOV), P651-660 ISSN: 1066-5099
Language: ENGLISH Document Type: ARTICLE
Abstract: The in vivo effects of pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF), a truncated molecule of recombinant human thrombopoietin modified with polyethylene glycol, were investigated in normal Balb/c mice. PEG-rHuMGDF was more potent in producing platelets and the dose-response curve was steeper compared with the case of the nonpegylated form of this molecule, Five

consecutive injections with PEG-rHuMGDF caused a dose-dependent increase in peripheral platelet counts with a peak on day 8. There was a dose-dependent rise in platelet counts on day 8 at daily doses from 0.333 to 30 mu g/kg. Intermediate doses of PEG-rHuMGDF (1.111 to 10 mu g/kg/day) caused a significant decrease in mean platelet volume, and conversely, higher doses of PEG-rHuMGDF (30 to 270 mu g/kg/day) induced a dose-dependent increase in mean platelet volume. There was a dose-dependent decrease in hemoglobin concentration with a minimum on day 8 but no significant reduction in reticulocyte counts following PEG-rHuMGDF administration. White blood cell counts were unchanged by PEG-rHuMGDF treatment. Marrow megakaryocyte size enlarged to 1.5-fold and the number of marrow megakaryocytes increased to sixfold by consecutive administration of PEG-rHuMGDF at 30 mu g/kg/day. A twofold increase in the number of marrow megakaryocytic progenitor cells (colony-forming units-megakaryocyte) was also observed. Marrow erythroid progenitor (colony-forming units-erythroid) counts decreased but splenic colony-forming units-erythroid, marrow and splenic erythro/myeloid progenitor cell counts, and splenic granulocyte/macrophage progenitor cell counts increased with PEG-rHuMGDF treatment. Marrow and splenic erythroid burst-forming cells were unchanged. These results indicate that PEG-rHuMGDF, a truncated molecule of thrombopoietin, is a potent stimulator for megakaryopoiesis and thrombopoiesis, and also affects the development of other hematopoietic cells in normal mice.

7/3,AB/125 (Item 32 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05385191 Genuine Article#: VT983 Number of References: 0 Title: ACQUIRED THROMBOCYTOPENIA ASSOCIATED WITH AN %ANTIBODY% THAT INTERFERES WITH THE %MPL% RECEPTOR AND/OR ITS LIGAND - A CASE-REPORT Author(s): NICHOL J; HORNKOHL A; BEST D; DUNN V; RICH D; HUNT P; YAFFE B Corporate Source: AMGEN INC/THOUSAND OAKS//CA/91320; KAISER PERMANENTE/ATLANTA//GA/00000
Journal: BLOOD, %1996%, V88, N10 (NOV 15), P2054
ISSN: 0006-4971
Language: ENGLISH Document Type: MEETING ABSTRACT

7/3,AB/126 (Item 33 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05327205 Genuine Article#: VQ324 Number of References: 69 Title: THROMBOPOIETIN - BIOLOGY, CLINICAL-APPLICATIONS, ROLE IN THE DONOR SETTING
Author(s): KUTER DJ
Corporate Source: MASSACHUSETTS GEN HOSP,HEMATOL ONCOL UNIT,COX 621,FRUIT ST/BOSTON//MA/02114; HARVARD UNIV,SCH MED/BOSTON//MA/00000 Journal: JOURNAL OF CLINICAL APHERESIS, %1996%, V11, N3, P149-159
ISSN: 0733-2459
Language: ENGLISH Document Type: ARTICLE
Abstract: Thrombopoietin (c-%Mpl% ligand) is the hematopoietic growth factor that is responsible for regulating the production of platelets from bone marrow megakaryocytes. This similar to 90 kd protein has recently been isolated and is comprised of an erythropoietin domain that is similar to 50% homologous to erythropoietin and a carbohydrate domain that is highly glycosylated and appears to stabilize the protein in the circulation. Thrombopoietin is produced in the liver and blood levels are determined by the mass of circulating platelets. However, there is no platelet ''sensor.'' Rather platelets contain high affinity thrombopoietin receptors that bind and remove thrombopoietin from the circulation and thereby directly determine circulating levels. In vitro thrombopoietin stimulates both early and late megakaryocyte precursors as well as some erythroid and multipotential progenitor cells. When administered to normal animals, it stimulates platelet production up to six-fold without affecting other lineages. However, when given to animals following chemotherapy or irradiation, it stimulates erythroid and myeloid as well as platelet recovery. Several different recombinant thrombopoietin proteins are now entering clinical trials in humans and all preliminary reports confirm a potent thrombopoietic stimulus and apparent lack of toxicity. Thrombopoietin shows great promise in preventing the thrombocytopenia associated with chemotherapy, bone marrow transplantation, and other acute or chronic thrombocytopenic disorders. In transfusion medicine, thrombopoietin may help mobilize peripheral blood progenitor cells, stimulate donors for plateletpheresis, and enhance platelet survival and function during storage. Many studies are currently underway in all these areas and should soon establish the role of thrombopoietin in clinical medicine. (C) 1996 Wiley-Liss, Inc.

7/3,AB/127 (Item 34 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05149404 Genuine Article#: VD260 Number of

References: 23 Title: REGULATION OF SERUM THROMBOPOIETIN LEVELS BY PLATELETS AND MEGAKARYOCYTES IN PATIENTS WITH APLASTIC-ANEMIA AND IDIOPATHIC THROMBOCYTOPENIC PURPURA
Author(s): ICHIKAWA N; ISHIDA F; SHIMODAIRA S; TAHARA T; KATO T; KITANO K Corporate Source: SHINSHU UNIV,SCH MED,DEPT INTERNAL MED 2,3-1-1 ASAHI/MATSUMOTO/NAGANO 390/JAPAN/; KIRIN BREWERY CO LTD,PHARMACEUT RES LAB/MAEBASHI/GUMMA/JAPAN/
Journal: THROMBOSIS AND HAEMOSTASIS, %1996%, V76, N2 (AUG), P156-160 ISSN: 0340-6245
Language: ENGLISH Document Type: ARTICLE
Abstract: To clarify the regulatory mechanism of thrombopoietin (TPO, c- %Mpl% ligand) in chronic thrombocytopenic conditions, we determined TPO levels in the sera of patients with aplastic anaemia (AA; n=26) and idiopathic thrombocytopenic purpura (ITP; n=32) by an enzyme-linked immunosorbent assay. Despite a similarity in platelet counts, serum TPO levels in the AA group were markedly higher than those in the ITP group: 20.41+/-9.71 fmol/ml (mean+/-SD) and 1.66+/-0.55 fmol/ml, respectively, both of which were significantly elevated compared to normal subjects (n=41; 1.22+/-0.37). in both groups, serum TPO level showed an inverse correlation with the platelet count. We determined the megakaryocyte volume using bone marrow clot section and found that it was markedly small in the AA group; while in the ITP group it was augmented with a correlation to serum TPO level, Our findings suggest that TPO levels may be regulated not only by platelets but also megakaryocytes in AA and ITP.

7/3,AB/128 (Item 35 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05141568 Genuine Article#: VC518 Number of References: 160 Title: PLATELETS AND COAGULATION - AN UPDATE
Author(s): BOUDREAUX MK
Corporate Source: AUBURN UNIV,COLL VET MED,DEPT PATHOBIOL,166 GREENE HALL/AUBURN//AL/36849
Journal: VETERINARY CLINICS OF NORTH AMERICA-SMALL ANIMAL PRACTICE, %1996% , V26, N5 (SEP), P1065&
ISSN: 0195-5616
Language: ENGLISH Document Type: REVIEW
Abstract: The latest developments in the areas of platelets and coagulation are presented and related to physiologic and pathologic processes. Platelet integrins, particularly alpha(IIb)beta(3), are discussed and their role in normal platelet aggregation, immune-mediated platelet destruction, and tumor cell metastasis are presented. New perspectives on coagulation pathways, mechanisms of antiphospholipid syndrome, and progress in hemophilia gene therapy are also discussed.

7/3,AB/129 (Item 36 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
(c) 2000 Inst for Sci Info. All rts. reserv.

05137473 Genuine Article#: VC742 Number of References: 20 Title: GPIIB/IIIA(+) SUBPOPULATION OF RAT MEGAKARYOCYTE PROGENITOR CELLS EXHIBITS HIGH RESPONSIVENESS TO HUMAN THROMBOPOIETIN
Author(s): KATO T; HORIE K; HAGIWARA T; MAEDA E; TSUMURA H; OHASHI H; MIYAZAKI H
Corporate Source: KIRIN BREWARY CO LTD,PHARMACEUT RES LAB,3 MIYAHARA CHO/TAKASAKI/GUMMA 37012/JAPAN/; KIRIN BREWARY CO LTD,PHARMACEUT RES LAB/TAKASAKI/GUMMA 37012/JAPAN/
Journal: EXPERIMENTAL HEMATOLOGY, %1996%, V24, N10 (AUG), P1209-1214 ISSN: 0301-472X
Language: ENGLISH Document Type: ARTICLE
Abstract: The recently cloned factor thrombopoietin (TPO) has been shown to exhibit megakaryocyte colony-stimulating activity in vitro. In this investigation, to further evaluate the action of TPO on megakaryocyte progenitor cells (colony-forming units-megakaryocyte [CFU-MK]), GpIIb/IIIa(+) and GpIIb/IIIa(-) populations of CFU-MK were prepared from rat bone marrow cells based on their reactivity with P55 %antibody%, a monoclonal %antibody% against rat GpIIb/IIIa, and their responsiveness to recombinant human TPO (rhTPO) and recombinant rat interleukin-3 (rrIL-3) was examined using a megakaryocyte colony-forming assay (Meg-CSA). rhTPO supported only megakaryocyte colony growth from both fractions in a dose-dependent fashion. The mean colony size observed with the GpIIb/IIIa(+) population was smaller than that seen with the GpIIb/IIIa(-) population. With the optimal concentration of either rhTPO or rrIL-3, similar numbers of megakaryocyte colonies were formed from the GpIIb/IIIa(+) population previously shown to be highly enriched for CFU-MK. In contrast, the maximum number of megakaryocyte colonies from the GpIIb/IIIa(-) population stimulated by rhTPO was only 24.2% of that achieved with rrIL-3. Morphologic analysis of rhTPO-promoted megakaryocyte colonies from the GpIIb/IIIa(+) population showed that the average colony size was smaller but that the mean diameter of individual megakaryocytes was larger than in megakaryocyte colonies promoted with rrIL-3. rhTPO plus rrIL-3, each at suboptimal concentrations, had an additive effect on proliferation of CFU-MK in the GpIIb/IIIa(+) fraction, whereas rhTPO plus murine

IL-6 or murine granulocyte-macrophage colony-stimulating factor (mG-M-CSF) modestly but significantly reduced megakaryocyte colony growth. These results indicate that TPO preferentially acts on GpIIb/IIIa+ late CFU-MK with lower proliferative capacity and interacts with some other cytokines in CFU-MK development.

7/3,AB/130 (Item 37 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05078437 Genuine Article#: TN924 Number of References: 27 Title: MODULATION OF PLATELET ACTIVATION IN-VITRO BY THROMBOPOIETIN
Author(s): KOJIMA H; HAMAZAKI Y; NAGATA Y; TODOKORO K; NAGASAWA T; ABE T Corporate Source: UNIV TSUKUBA,INST CLIN CHEM,DIV HEMATOL/TSUKUBA/IBARAKI 305/JAPAN/; RIKEN,TSUKUBA LIFE SCI CTR/TSUKUBA/IBARAKI/JAPAN/ Journal: THROMBOSIS AND HAEMOSTASIS, %1995%, V74, N6 (DEC), P1541-1545 ISSN: 0340-6245
Language: ENGLISH Document Type: ARTICLE
Abstract: Effect of human recombinant thrombopoietin (TPO) on platelet activation in vitro was studied. Although TPO by itself did not cause platelet aggregation, it upregulated ADP-induced aggregation, especially the second wave of aggregation. This effect was dose-dependent for up to 5 ng/ml of TPO. When platelets were activated by epinephrine, collagen, or alpha-thrombin, similar effect was observed. However, TPO did not affect A23187- or PMA-induced aggregation, suggesting that TPO may have modulated the signal transduction pathway upstream of inositol 1,4,5-trisphosphate and diacylglycerol production. TPO also upregulated thrombin-induced alpha-granule secretion. To clarify the involvement of protein tyrosine phosphorylation, platelets were activated by TPO and/or suboptimal concentration of ADP, then tyrosine phosphorylation was detected by immunoblot analysis, using anti-phosphotyrosine monoclonal %antibody%. TPO by itself caused significant tyrosine phosphorylation of 146, 130, 122, 108, 97, 94, and 88 kDa proteins. Further, by using %antibodies% against signal transduction molecules, for immunoprecipitation, we observed the significant tyrosine phosphorylation in Jak2 and Tyk2 molecules after TPO-stimulation. The results of the present experiment clearly indicate that TPO directly activated platelets and modulated intracellular signal transduction pathway.

7/3,AB/131 (Item 38 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05066932 Genuine Article#: TM897 Number of References: 45 Title: EXPERIMENTAL ANTHRAX VACCINES - EFFICACY OF ADJUVANTS COMBINED WITH PROTECTIVE ANTIGEN AGAINST AN AEROSOL BACILLUS-ANTHRACIS SPORE CHALLENGE IN GUINEA-PIGS
Author(s): IVINS B; FELLOWS P; PITT L; ESTEP J; FARCHAUS J; FRIEDLANDER A; GIBBS P Corporate Source: USA,MED RES INST INFECT DIS,DIV BACTERIOL,FT DETRICK/FREDERICK//MD/21702; USA,MED RES INST INFECT DIS,DIV APPL RES,FT DETRICK/FREDERICK//MD/21702; USA,MED RES INST INFECT DIS,DIV BIOMETR & INFORMAT MANAGEMENT,FT DETRICK/FREDERICK//MD/21702 Journal: VACCINE, %1995%, V13, N18 (DEC), P1779-1784 ISSN: 0264-410X
Language: ENGLISH Document Type: ARTICLE
Abstract: The efficacy of several human anthrax vaccine candidates comprised of different adjuvants together with Bacillus anthracis protective antigen (PA) was evaluated in guinea pigs challenged by an aerosol of virulent B. anthracis spores. The most efficacious vaccines tested were formulated with PA plus monophosphoryl lipid A (%MPL%) in a squalene/lecithin/Tween 80 emulsion (SLT) and PA plus the saponin QS-21. The PA+%MPL% in SLT vaccine, which was lyophilized and then reconstituted before use, demonstrated strong protective immunogenicity, even after storage for 2 years at 4 degrees C. The %MPL% component was required for maximum efficacy of the vaccine. Eliminating lyophilization of the vaccine did not diminish its protective efficacy. No significant alteration in efficacy was observed when PA was dialyzed against different buffers before preparation of vaccine. PA+%MPL% in SLT proved superior in efficacy to the licensed United States human anthrax vaccine in the guinea pig model.

7/3,AB/132 (Item 39 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05028532 Genuine Article#: TH910 Number of References: 0 Title: IMMUNE THROMBOCYTOPENIA ASSOCIATED WITH AN %ANTIBODY% TO C-%MPL%, THE THROMBOPOIETIN (TPO) RECEPTOR
Author(s): MALLOY B; NOEL P; EATON D; SOLBERG L Corporate Source: GENENTECH INC/S SAN FRANCISCO//CA/94080; MAYO CLIN SCOTTSDALE/SCOTTSDALE//AZ/00000; MAYO CLIN JACKSONVILLE/JACKSONVILLE//FL/32224 Journal: BLOOD, %1995%, V86, N10 (NOV 15), P1102 ISSN: 0006-4971
Language: ENGLISH Document Type: MEETING ABSTRACT

7/3,AB/133 (Item 40 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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05016505 Genuine Article#: UZ413 Number of References: 13 Title: SERUM THROMBOPOIETIN AND PLASMA GLYCOCALICIN CONCENTRATIONS AS USEFUL DIAGNOSTIC MARKERS IN THROMBOCYTOPENIC DISORDERS Author(s): KUNISHIMA S; TAHARA T; KATO T; KOBAYASHI S; SAITO H; NAOE T Corporate Source: NAGOYA UNIV,BRANCH HOSP,DEPT INTERNAL MED,HIGASHI KU,1-1-20 DAIKO MINAMI/NAGOYA/AICHI 461/JAPAN/; NAGOYA UNIV,BRANCH HOSP,DEPT INTERNAL MED,HIGASHI KU/NAGOYA/AICHI 461/JAPAN/; MEIJO HOSP,DEPT CENT CLIN LAB,NAKA KU/NAGOYA/AICHI 460/JAPAN/; NAGOYA UNIV,SCH MED,DEPT INTERNAL MED 1,SHOWA KU/NAGOYA/AICHI 466/JAPAN/; KIRIN BREWERY CO LTD,PHARMACEUT RES LAB/MAEBASHI/GUMMA 371/JAPAN/ Journal: EUROPEAN JOURNAL OF HAEMATOLOGY, %1996%, V57, N1 (JUL), P68-71 ISSN: 0902-4441
Language: ENGLISH Document Type: ARTICLE
Abstract: Using enzyme-linked immunosorbent assays, we measured the concentrations of serum thrombopoietin (TPO) and plasma glycocalicin, a proteolytic fragment of platelet glycoprotein Iba, in 13 patients with myelodysplastic syndrome (MDS), aplastic anaemia (AA) or idiopathic thrombocytopenic purpura (ITP). In the patients with AA or MDS, the TPO concentrations were remarkably increased, and their glycocalicin concentrations were decreased compared with the normal control individuals. In the patients with ITP, however, the TPO and glycocalicin levels were not changed as much as in the AA/MDS patients in spite of the same degree of thrombocytopenia. During immunosuppressive treatment of ITP patients, there was an inverse relationship between the level of TPO and the platelet count. Thus, measurements of TPO and glycocalicin levels are useful for the diagnosis of thrombocytopenia, and our results from ITP patients did not support the model which suggested the simple feedback regulation of TPO in thrombocytopenia.

7/3,AB/134 (Item 41 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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04971343 Genuine Article#: UX319 Number of References: 43 Title: DIMERIZATION OF A CHIMERIC CD4-INTERFERON-ALPHA RECEPTOR RECONSTITUTES THE SIGNALING EVENTS PRECEDING STAT PHOSPHORYLATION Author(s): KRISHNAN K; YAN H; LIM JTE; KROLEWSKI JJ
Corporate Source: COLUMBIA UNIV COLL PHYS & SURG,DEPT PATHOL/NEW YORK//NY/10032; COLUMBIA UNIV COLL PHYS & SURG,DEPT PATHOL/NEW YORK//NY/10032; COLUMBIA UNIV COLL PHYS & SURG,CTR CANC/NEW YORK//NY/10032
Journal: ONCOGENE, %1996%, V13, N1 (JUL 4), P125-133 ISSN: 0950-9232
Language: ENGLISH Document Type: ARTICLE
Abstract: Interferon-alpha induces the rapid tyrosine phosphorylation of a number of molecules, including the cognate receptors, JAK-family kinases (Jak1 and tyk2), and latent transcription factors (STATs 1 and 2), Here, we describe the use of chimeric molecules composed of the extracellular domain of CD4 fused to the intracellular domain of the interferon-alpha receptor subunit 1 (IFNaR1), %Antibody% mediated crosslinking dimerizes the transfected chimeras, activates tyk2 and induces a tyk2-dependent tyrosine phosphorylation of the intracellular domain of the chimera, We further define the major site of IFNaR1 phosphorylation, and show that phosphorylation of this site is required for association with STAT2, Finally, we show that homodimerization of IFNaR1 is not sufficient to activate the STATs, suggesting a role for the IFNaR2 subunit and Jak1 in the transduction of the interferon-alpha signal.

7/3,AB/135 (Item 42 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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04951224 Genuine Article#: UU824 Number of References: 17 Title: A SENSITIVE SANDWICH ELISA FOR MEASURING THROMBOPOIETIN IN HUMAN SERUM - SERUM THROMBOPOIETIN LEVELS IN HEALTHY-VOLUNTEERS AND IN PATIENTS WITH HEMATOPOIETIC DISORDERS
Author(s): TAHARA T; USUKI K; SATO H; OHASHI H; MORITA H; TSUMURA H; MATSUMOTO A; MIYAZAKI H; URABE A; KATO T
Corporate Source: KIRIN BREWERY CO LTD,PHARMACEUT RES LAB,1-2-2 SOUJA MACHI/MAEBASHI/GUMMA 371/JAPAN/; KIRIN BREWERY CO LTD,PHARMACEUT DEV LAB/MAEBASHI/GUMMA 371/JAPAN/; KANTO TEISHIN HOSP,DIV HAEMATOL/TOKYO//JAPAN/
Journal: BRITISH JOURNAL OF HAEMATOLOGY, %1996%, V93, N4 (JUN), P783-788 ISSN: 0007-1048
Language: ENGLISH Document Type: ARTICLE
Abstract: A sensitive sandwich enzyme-linked immunosorbent assay (ELISA) has been established to estimate serum thrombopoietin (TPO) concentrations in

healthy volunteers and patients with haemopoietic disorders. The ELISA uses a mouse monoclonal %antibody% (Ab) as the capture Ab and a biotinylated rabbit polyclonal Ab as the detector. The ELISA was reproducible, highly sensitive and specific for human TPO. The coefficients of intra- and interassay variation were from 3.0% to 4.9% and from 5.9% to 6.1%, respectively. The quantitative limit of the ELISA was 0.09 fmol/ml in serum. The quantitative limit was lower than the normal level. The dose-response curves of serum samples from healthy volunteers and patients with haemopoietic disorders were parallel to the standard curves. The ELISA did not cross-react with a variety of blood components and cytokines to produce false-positive results. The serum TPO concentrations from 29 normal males and 21 females were 0.79 +/- 0.35 and 0.70 +/- 0.26 fmol/ml, respectively. Serum TPO levels in patients with aplastic anaemia (AA). acute lymphocytic leukaemia (ALL) and essential thrombocythaemia (ET) were measured using the ELISA. The serum TPO levels in the patients with ET (n = 6, 2.80 +/- 1.55 fmol/ml) were higher than the normal level. The patients with AA (n = 7, 18.53 +/- 12.37 fmol/ml) and ALL (n = 5, 10.36 +/- 5.57 fmol/ml) had significantly higher serum TPO levels than normal individuals. These results indicate that the ELISA specific to TPO should prone useful in measuring the TPO concentration in serum samples.

7/3,AB/136 (Item 43 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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04824327 Genuine Article#: UK879 Number of References: 39 Title: HUMAN THROMBOPOIETIN LEVELS ARE HIGH WHEN THROMBOCYTOPENIA IS DUE TO MEGAKARYOCYTE DEFICIENCY AND LOW WHEN DUE TO INCREASED PLATELET DESTRUCTION
Author(s): EMMONS RVB; REID DM; COHEN RL; MENG G; YOUNG NS; DUNBAR CE; SHULMAN NR
Corporate Source: NHLBI,HEMATOL BRANCH,NIH,9000 ROCKVILLE PIKE,BLDG 10,ROOM 7C103/BETHESDA//MD/20892; NIDDKD,CLIN HEMATOL BRANCH,NATL INST HLTH/BETHESDA//MD/20892; GENENTECH INC/S SAN FRANCISCO//CA/94080 Journal: BLOOD, %1996%, V87, N10 (MAY 15), P4068-4071
ISSN: 0006-4971
Language: ENGLISH Document Type: ARTICLE
Abstract: Thrombopoietin (TPO), the ligand for c-%mpl%, stimulates proliferation of committed megakaryocytic progenitors and induces maturation of megakaryocytes. To better understand factors regulating TPO levels, we measured blood levels of TPO in patients with impaired platelet production due to aplastic anemia (AA) and with platelet destructive disorders, including idiopathic thrombocytopenic purpura (ITP), posttransfusion purpura (PTP), drug purpura (DP), and X-linked thrombocytopenia (XLTP). The TPO receptor capture enzyme immunoassay (EIA) used had a detection limit of similar to 150 to 200 pg/mL. TPO was undetectable in 88 of 89 normal individuals. Eighteen of 19 patients with AA and a mean platelet count (MPC) of 18,000/mu L (2,000 to 61,000/mu L) had markedly elevated TPO levels (mean, 1,467 pg/mL; range, 597 to 3,834 pg/mL). Eight AA patients who responded to immunosuppressive therapy with their MPC increasing to 140,000/mu L (92,000 to 175,000/mu L) had substantial decreases in TPO (mean, 440 pg/mL; range, 193 to 771 pg/mL). Initial TPO levels did not differ significantly between responders and nonresponders. In contrast, all 21 patients with ITP and an MPC of 16,000/mu L (1,000 to 51,000/mu L) had undetectable TPO levels, as did 6 patients with acute PTP or DP and 2 patients with XLTP. Megakaryocyte mass, reflected in the rate of platelet production, appears to be the major determinant of TPO levels in thrombocytopenic patients rather than circulating platelet levels per se. Measurement of serum TPO may be useful in differentiating thrombocytopenias due to peripheral destruction from those due to thrombopoietic failure.

7/3,AB/137 (Item 44 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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04677469 Genuine Article#: UA957 Number of References: 42 Title: DEFICIENCIES IN PROGENITOR CELLS OF MULTIPLE HEMATOPOIETIC LINEAGES AND DEFECTIVE MEGAKARYOCYTOPOIESIS IN MICE LACKING THE THROMBOPOIETIN RECEPTOR C-%MPL%
Author(s): ALEXANDER WS; ROBERTS AW; NICOLA NA; LI RL; METCALF D Corporate Source: PO ROYAL MELBOURNE HOSP,WALTER & ELIZA HALL INST MED RES/MELBOURNE/VIC 3050/AUSTRALIA/
Journal: BLOOD, %1996%, V87, N6 (MAR 15), P2162-2170
ISSN: 0006-4971
Language: ENGLISH Document Type: ARTICLE
Abstract: Mice with a null mutation in the thrombopoietin (TPO) receptor c- %Mpl% were generated by gene targeting. c-%mpl%-deficient mice developed normally but were deficient in megakaryocytes and severely thrombocytopenic, The hematocrit and numbers of mature circulating leukocytes were normal in %mpl%(-/-) mice, as was the distribution of morphologically identifiable precursors in hematopoietic tissues, Bone marrow and spleen cells of adult %mpl%(-/-) mice lacked specific binding sites for TPO,

were unresponsive to TPO in culture, and displayed a marked deficiency in progenitor cells with megakaryocytic potential, Significantly, total hematopoietic progenitor cell numbers were also reduced in %mpl%(-/-) mice, including multipotential, blast cell, and committed progenitors of multiple lineages. The megakaryocyte deficiency was evident as early as 14 days of gestation in %mpl% -deficient mice, although reductions in progenitor cell numbers arose only later in development. The data suggest that the critical function of c-%Mpl% signalling in megakaryocytopoiesis is in maintenance of mature megakaryocyte numbers through control of progenitor cell proliferation and maturation, Moreover, our results also imply an important role for TPO and c-%Mpl% in the production of primitive pluripotent progenitor cells as well as progenitor cells committed to nonmegakaryocytic lineages. (C) 1996 by The American Society of Hematology.

7/3,AB/138 (Item 45 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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04593900 Genuine Article#: TV689 Number of References: 26 Title: EFFECTS OF MONOPHOSPHORYLLIPID-A ON THE IMMUNIZATION OF MICE WITH KEYHOLE LIMPET HEMOCYANIN-GANGLIOSIDE AND MURAMYLDIPEPTIDE-GANGLIOSIDE GFPT1 CONJUGATES
Author(s): JENNEMANN R; BAUER BL; SCHMIDT R; ELSASSER HP; WIEGANDT H Corporate Source: UNIV MARBURG,INST PHYSIOL CHEM,KARL VON FRISCH STR 1/D-35043 MARBURG//GERMANY/; UNIV MARBURG,INST PHYSIOL CHEM/D-35043 MARBURG//GERMANY/; UNIV MARBURG,ZENTRUM OPERAT MED 2,ABT NEUROCHIRURG/W-3550 MARBURG//GERMANY/; UNIV MARBURG,INST ZYTOBIOL/W-3550 MARBURG//GERMANY/
Journal: JOURNAL OF BIOCHEMISTRY, %1996%, V119, N2 (FEB), P378-384 ISSN: 0021-924X
Language: ENGLISH Document Type: ARTICLE
Abstract: Since it was considered that an active immunization against ganglioside Gfpt1 (IV(2)Fuc-, II(3)NeuAc-Gg(4)Cer) expressed by human small cell lung cancer cells may be beneficial in the treatment of this neoplasm in humans, an optimal mode of vaccination in model mice was investigated. A novel Gfpt1-muramyldipeptide conjugate (Gfpt1-MDP) was synthesized, Its ganglioside carbohydrate-directed immunogenicity in mice as measured by serum %antibody% titers was comparable to that of the previously described Gfpt1-keyhole limpet hemocyanin conjugate (Gfpt1-KLH). Similar immunogenicity was displayed by free Gfpt1 in muramyldipeptide-phosphoethanolamine-containing phosphatidyl-choline, -serine (PC, PS) liposomes. Immunization with Gfpt1-vaccines in the presence of monophosphoryllipid A (%MPL%), in general, raised titers of anti-Gfpt1 %antibodies% effectively, Immunization with PC, PS-liposomes containing unconjugated Gfpt1 and %MPL% stimulated the highest titers observed, thereby effectively preventing tumor growth in Balbc nu/nu-mice challenged with human small cell lung cancer cells, However, there was a strong crossreaction of these and most other sera with the structurally related and widely distributed ganglioside Gtet1 (II(3)NeuAc-Gg(4)Cer). Only immunization with Gfpt1-KLH conjugate in the presence of %MPL% stimulated selectively high anti-Gfpt1 %antibody% titers showing comparably low crossreactivity to ganglioside Gtet1.

7/3,AB/139 (Item 46 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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04484450 Genuine Article#: TF798 Number of References: 32 Title: ESTABLISHMENT AND CHARACTERIZATION OF A NEW HUMAN MESOTHELIOMA CELL-LINE (T-85) FROM MALIGNANT PERITONEAL MESOTHELIOMA WITH REMARKABLE THROMBOCYTOSIS
Author(s): TANGE T; HASEGAWA Y; OKA T; SUNAGA S; HIGASHIHARA M; MATSUO K; MIYAZAKI H; SHIMOSAKA A; OKANO A; TODOKORO K; ISHIKAWA T; MACHINAMI R Corporate Source: UNIV TOKYO,FAC MED,DEPT PATHOL,BUNKYO KU,7-3-1 HONGO/TOKYO 113//JAPAN/; UNIV TOKYO,FAC MED,DEPT INTERNAL MED,BUNKYO KU/TOKYO 113//JAPAN/; KYUSHU UNIV,FAC DENT,DEPT ORAL PATHOL/FUKUOKA 812//JAPAN/; KIRIN BREWERY CO LTD,PHARMACEUT RES LAB/MAEBASHI/GUMMA/JAPAN/; AJINOMOTO CO INC,CENT RES LABS/KAWASAKI/KANAGAWA 210/JAPAN/; INST PHYS & CHEM RES,TSUKUBA LIFE SCI CTR/TSUKUBA/IBARAKI/JAPAN/
Journal: PATHOLOGY INTERNATIONAL, %1995%, V45, N11 (NOV), P791-800 ISSN: 1320-5463
Language: ENGLISH Document Type: ARTICLE
Abstract: A mesothelioma cell line, termed T-85, was established from a patient with malignant peritoneal mesothelioma and remarkable thrombocytosis (1.4 x 10(6)/mm(3)). Electron microscopically, two types of mesothelioma cells have been characterized; the major type of cells with dense-cored granules in the cytoplasm and the minor one with evenly dense granules, Immunologically, the cells showed staining for interleukin-6 (IL-6), cytokeratin, collagen type IV, vimentin, laminin, fibronectin and Factor VIII-related

antigen. Quantitation by ELISA revealed a high concentration of IL-6 in T-85 cell culture supernatants. RT-polymerase chain reaction of T-85 cells showed two positive bands of cDNA at 628 and 251 base pairs indicating the constitutive expression of IL-6 and IL-6 receptor mRNA, Moreover, prominent pro-platelet process formation activity in T-85 cell culture supernatants indicated the presence of a thrombopoietic activity due mainly to IL-6 but not the c-%Mpl% ligand or erythropoietin, However, the fact that 15% of PPF activity remained in the supernatants treated with anti-IL-6 %antibody% indicated the presence of another thrombopoietic substance, T-85 is so far the first mesothelioma cell line derived from a case with remarkable thrombocytosis.

7/3,AB/140 (Item 47 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03908110 Genuine Article#: QR968 Number of References: 37 Title: THROMBOPOIETIN, THE %MPL% LIGAND, IS ESSENTIAL FOR FULL MEGAKARYOCYTE DEVELOPMENT
Author(s): KAUSHANSKY K; BRODUY VC; LIN N; JORGENSEN MJ; MCCARTY J; FOX N; ZUCKERFRANKLIN D; LOFTONDAY C
Corporate Source: UNIV WASHINGTON,DIV HEMATOL RM10/SEATTLE//WA/98195; NYU,DEPT MED/NEW YORK//NY/10016; ZYMOGENET INC/SEATTLE//WA/98102 Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, %1995%, V92, N8 (APR 11), P3234-3238 ISSN: 0027-8424
Language: ENGLISH Document Type: ARTICLE
Abstract: The development of megakaryocytes (MKs) from their marrow precursors is one of the least understood aspects of hematopoiesis. Current models suggest that early-acting MK colony-stimulating factors, such as interleukin (IL) 3 or c-kit ligand, are required for expansion of hematopoietic progenitors into cells capable of responding to late-acting MK potentiators, including IL-6 and IL-11. Recently, the %Mpl% ligand, or thrombopoietin (Tpo), has been shown to display both MK colony-stimulating factor and potentiator activities, at potencies far greater than that of other cytokines. In light of these findings, we tested the hypothesis that Tpo is absolutely necessary for MK development. In this report we demonstrate that neutralizing the biological activity of Tpo eliminates MK formation in response to c-kit ligand, IL-6, and IL-11, alone and in combination, but that these reagents only partially reduce MK formation in the presence of combinations of cytokines including IL-3. However, despite the capacity of IL-3 to support the proliferation and initial stages of MK differentiation, elimination of Tpo prevents the full maturation of IL-3-induced MK. These data indicate that two populations of MK progenitors can be identified: one that is responsive to IL-3 but can fully develop only in the presence of Tpo and a second that is dependent on Tpo for both proliferation and differentiation. Thus, our results strongly suggest that Tpo is the primary regulator of MK development and platelet production.

7/3,AB/141 (Item 48 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03727043 Genuine Article#: QB546 Number of References: 52 Title: THE MPI RECEPTOR IS EXPRESSED IN THE MEGAKARYOCYTIC LINEAGE FROM LATE PROGENITORS TO PLATELETS
Author(s): DEBILI N; WENDLING F; COSMAN D; TITEUX M; FLORINDO C; DUSANTERFOURT I; SCHOOLEY K; METHIA N; CHARON M; NADOR R; BETTAIEB A; VAINCHENKER W
Corporate Source: INST GUSTAVE ROUSSY,INSERM,U362,PAVILLON RECH 1,39 RUE CAMILLE DESMOULINS/F-94805 VILLEJUIF//FRANCE/; INST GUSTAVE ROUSSY,INSERM,U362/F-94805 VILLEJUIF//FRANCE/; HOP COCHIN,INSERM,U363/PARIS//FRANCE/; IMMUNEX CORP/SEATTLE//WA/00000; HOP HENRI MONDOR,CTR DEPT TRANSFUS/CRETEIL//FRANCE/
Journal: BLOOD, %1995%, V85, N2 (JAN 15), P391-401
ISSN: 0006-4971
Language: ENGLISH Document Type: ARTICLE
Abstract: The %Mpl% receptor (%Mpl%-R) is a cytokine receptor belonging to the hematopoietin receptor superfamily for which a ligand has been recently characterized. To study the lineage distribution of %Mpl%-R in normal hematopoietic cells, we developed a monoclonal %antibody% (designated M1 MoAb) by immunizing mice with a soluble form of the human %Mpl%-R protein. With few exceptions, %Mpl%-R was detected by indirect immunofluorescent analysis on all human leukemic hematopoietic cell lines with pluripotential and megakaryocytic phenotypes, but not on other cell lines. By immunoprecipitation and immunoblotting, M1 MoAb recognized a band at 82 to 84 kD corresponding to the expected size of the glycosylated receptor. Among normal hematopoietic cells, M1 MoAb strongly stained megakaryocytes (MK) and %Mpl%-R was detected on platelets by indirect immunofluorescence staining or immunoblotting. On purified CD34(+) cells, less than 2% of the population was stained, but the labeling was weak and just above the threshold of detection. However, dual-labeling with the M1 and antiplatetet glycoprotein MoAbs showed that

most %Mpl%-R(+)/CD34(+) cells coexpressed CD41a, CD61, or CD42a, suggesting that cell surface appearance of %Mpl%-R and platelet glycoproteins could be coordinated. M1-positive and M1-negative subsets were sorted from purified CD34(+) cell populations. Colony assays showed that the absolute number of hematopoietic progenitors was extremely low and no primitive progenitors were present in the CD34(+)/%Mpl%-R(+) fraction. However, this cell fraction was significantly enriched in low proliferative colony-forming units-MK. When the CD34(+)/%Mpl%-R(+) fraction was grown in liquid culture containing human aplastic serum and a combination of growth factors, mature MK were seen as early as day 4, whereas the predominant cell population was erythroblasts on day 8. Similar data were also obtained with the CD34(+)/%Mpl%-R(-)fraction with, however, a delay in the time of appearance of both MK and erythroblasts. In conclusion, %Mpl%-R is a cytokine receptor restricted to the MK cell lineage. Its expression is low on CD34(+) cells and these cells mainly correspond to late MK progenitors and transitional cells. These data indicate that the action of the %Mpl%-R ligand might predominate during the late stages of human MK differentiation. (C) 1995 by The American Society of Hematology.

7/3,AB/142 (Item 49 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03640492 Genuine Article#: PU149 Number of References: 89 Title: THE STRUCTURE, BIOLOGY AND POTENTIAL THERAPEUTIC APPLICATIONS OF RECOMBINANT THROMBOPOIETIN
Author(s): LOK S; FOSTER DC
Corporate Source: ZYMOGENET CORP,CYTOKINE RES CTR,1201 EASTLAKE AVE E/SEATTLE//WA/98102
Journal: STEM CELLS, %1994%, V12, N6 (NOV), P586-598
ISSN: 1066-5099
Language: ENGLISH Document Type: REVIEW
Abstract: Platelets, an integral component of hemostasis, are produced by megakaryocytes derived from the differentiation of pluripotent stem cells in the bone marrow or spleen. After decades of study, the regulation of this process is still not well understood. However, the recent cloning and characterization of thrombopoietin, a ligand for the receptor encoded by the c-%mpl% proto-oncogene, provides new insights into the humoral regulation of megakaryocytopoiesis and platelet production. Consistent with the proposed role as a major physiological regulator of megakaryocytopoiesis, thrombopoietin has potent effects on megakaryocytopoiesis in vitro and in vivo. In addition to the original supposition that thrombopoietin functions as a late-acting megakaryocyte maturation factor, recombinant thrombopoietin proves also to be a potent stimulator of hematopoietic progenitor cells, inducing them to undergo proliferation and differentiation into megakaryocytic colonies. When administered to mice, thrombopoietin causes an increase in peripheral platelet numbers to previously unattainable levels within a few days. Studies of the efficacy of thrombopoietin are underway. It is envisaged that this new cytokine will have widespread applications as a therapeutic agent for the management of bleeding due to thrombocytopenias, in particular those resulting from cancer chemo- or irradiation therapy.

7/3,AB/143 (Item 50 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03613414 Genuine Article#: PR430 Number of References: 24 Title: COLOCALIZATION OF PLACENTAL LACTOGEN-I, PLACENTAL LACTOGEN-II, AND PROLIFERIN IN THE MOUSE PLACENTA AT MIDPREGNANCY
Author(s): YAMAGUCHI M; OGREN L; ENDO H; SOARES MJ; TALAMANTES F Corporate Source: UNIV CALIF SANTA CRUZ,DEPT BIOL/SANTA CRUZ//CA/95064; UNIV CALIF SANTA CRUZ,DEPT BIOL/SANTA CRUZ//CA/95064; UNIV KANSAS,MED CTR,DEPT PHYSIOL/KANSAS CITY//KS/66103
Journal: BIOLOGY OF REPRODUCTION, %1994%, V51, N6 (DEC), P1188-1192 ISSN: 0006-3363
Language: ENGLISH Document Type: ARTICLE
Abstract: This study was undertaken to determine whether mouse placental lactogen (%mPL%)-I, %mPL%-II, and proliferin (PLF) are expressed by the same population of placental giant cells at midpregnancy. Tissue sections from Day 9 of pregnancy were analyzed by double immunofluorescence staining. Sections were stained for PLF by use of a rhodamine-conjugated second %antibody%, and for %mPL%-I or %mPL%-II by use of a fluorescein-conjugated second %antibody%. All three proteins were present in most of the same giant cells. The distribution of %mPL% -I and PLF among giant cells in vitro was also examined. When placental cells from Day 7 of pregnancy were cultured for 5 days, > 90% of the cells that immunostained for %mPL%-I also immunostained for PLF on the first 3 days of culture. Thereafter, the percentage of cells that contained both proteins declined rapidly while the percentage that contained only PLF increased, suggesting continued differentiation of the cells in vitro. These data demonstrate that the same trophoblast giant cells express %mPL%-I, %mPL%-II, and PLF simultaneously at midpregnancy, suggesting that their gestational profiles in maternal blood during this period result at least

partly from changes in gene expression in one population of cells and not from differentiation of several subsets of giant cells, each expressing only one member of the gene family.

7/3,AB/144 (Item 51 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03343528 Genuine Article#: NY176 Number of References: 30 Title: EFFICACY OF TUMOR-CELL VACCINE AFTER INCORPORATING MONOPHOSPHORYL LIPID-A (%MPL%) IN TUMOR-CELL MEMBRANES CONTAINING TURNER-ASSOCIATED GANGLIOSIDE
Author(s): RAVINDRANATH MH; BRAZEAU SM; MORTON DL
Corporate Source: JOHN WAYNE CANC INST,GLYCOLIPID IMMUNOTHERAPY LAB,2200 SANTA MONICA BLVD/SANTA MONICA//CA/90404
Journal: EXPERIENTIA, %1994%, V50, N7 (JUL 15), P648-653
ISSN: 0014-4754
Language: ENGLISH Document Type: ARTICLE
Abstract: Murine B16 melanoma expresses the ganglioside GM(3). GM(3) shed from tumor cells is immunosuppressive and promotes tumor growth(1). Reduction or elimination of the shed GM(3) could be therapeutic, and the anti-GM(3) %antibodies% may reduce and clear the shed ganglioside. To test this hypothesis, mice were challenged with tumor cells, with or without inducing anti-GM(3) %antibody% response. Since gangliosides are poor immunogens and T-cell independent antigens, an adjuvant (monophosphoryl lipid A (%MPL%), a non-toxic lipid A of Salmonella), directed against B-cells, was employed. %MPL% was incorporated onto liposomes and into the surface membrane of B16 mouse melanoma cells; both are rich in GM(3). C57BL/6J mice immunized with %MPL%-liposomes or %MPL%-B16 cells responded with elevated levels of anti-GM(3) IgM. Non-immunized mice or mice immunized with B16 cells alone or ganglioside GM(3) alone (without %MPL%) elicited poor anti-GM(3) IgM response, confirming the GM(3)'s immunologic crypticity and %MPL%'s immunopotentiating effect. %MPL%'s immunopotentiating effect was improved by coupling it to melanoma cell membranes. C57BL/6J mice were immunized with irradiated B16 alone or %MPL% alone or %MPL%-conjugated irradiated B16. After three weekly immunizations, each mouse received a challenge dose of viable syngeneic B16. Neither %MPL% alone nor B16 alone had a significant effect on tumor growth or host survival; however, administration of %MPL%-conjugated B16 cells significantly prevented tumor growth and prolonged survival. Our results indicate that %MPL%-incorporated B16 cells augment the anti-GM(3) IgM response, which may reverse GM(3)-induced immunosuppression by eliminating tumor-derived GM(3), and restore immunocompetence.

7/3,AB/145 (Item 52 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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03017631 Genuine Article#: MW700 Number of References: 58 Title: AMMONIA AFFECTS THE GLYCOSYLATION PATTERNS OF RECOMBINANT MOUSE PLACENTAL LACTOGEN-1 BY CHINESE-HAMSTER OVARY CELLS IN A PH-DEPENDENT MANNER
Author(s): BORYS MC; LINZER DIH; PAPOUTSAKIS ET
Corporate Source: NORTHWESTERN UNIV,DEPT CHEM ENGN/EVANSTON//IL/60208; NORTHWESTERN UNIV,DEPT CHEM ENGN/EVANSTON//IL/60208; NORTHWESTERN UNIV,DEPT BIOCHEM MOLEC BIOL & CELL BIOL/EVANSTON//IL/60208
Journal: BIOTECHNOLOGY AND BIOENGINEERING, %1994%, V43, N6 (MAR 15), P 505-514
ISSN: 0006-3592
Language: ENGLISH Document Type: ARTICLE
Abstract: The N-linked glycosylation of the recombinant protein mouse placental lactogen-I (%mPL%-I) expressed by Chinese hamster ovary (CHO) cells under nongrowth conditions was inhibited by increasing levels of ammonium chloride (3 and 9 mM) in a serum-free, protein expression medium. The effect of ammonia on glycosylation was dependent on the extracellular pH (pH(e)). In media containing 0 and 9 mM ammonium chloride, the percentage of the most heavily glycosylated forms of secreted %mPL%-I decreased from ca. 90% to ca. 25% at pH(e) 8.0, and from ca. 90% to ca. 65% at pH(e) 7.6, respectively. However, at pH(e) 7.2, the most heavily glycosylated forms of secreted %mPL%-I decreased from ca. 90% to ca. 80% in media containing 0 and 9 mM ammonium chloride, respectively. Inhibition of %mPL%-I glycosylation was found to correlate with the calculated concentration of the ammonia species (NH3). Control experiments showed that the ammonia effect on %mPL%-I glycosylation could not be attributed to increased chloride concentration or osmolarity, or to extracellular events after secretion of the recombinant protein into the supernatant. Ammonium chloride, 9 mM, inhibited the expression rate of %mPL%-I by CHO cells at low pH(e). (C) 1994 John Wiley and Sons, Inc.

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DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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02906509 Genuine Article#: MN577 Number of References: 21 Title: THE INFLUENCE OF MONOPHOSPHORYL LIPID A (%MPL%((TM))) ON ERYTHROCYTE AUTOANTIBODY FORMATION
Author(s): HRABA T; BAKER PJ; TAYLOR CE; FAUNTLEROY MB; STASHAK PW Corporate Source: NIAID,IMMUNOGENET LAB,TWINBROOK 2 RES FACIL,12441 PARKLAWN DR/ROCKVILLE//MD/20852; NIAID,IMMUNOGENET LAB,TWINBROOK 2 RES FACIL/ROCKVILLE//MD/20852
Journal: IMMUNOBIOLOGY, %1993%, V189, N5 (DEC), P448-456
ISSN: 0171-2985
Language: ENGLISH Document Type: ARTICLE
Abstract: The onset and the amount of erythrocyte autoantibodies induced by the injection of C57BL/6N mice with rat red blood cells (RRBC) were hastened and increased, respectively, after the administration of monophosphoryl lipid A (MpLT(TM)); this was not the case for similarly treated BALB/cAnN mice, which make a lower autoantibody response after immunization with RRBC. The transfer of spleen cells from donor C57BL/6N mice immunized with RRBC suppressed autoantibody formation in recipient mice subsequently immunized with RRBC; however, treatment with %MpL%(TM) prevented neither the induction nor the expression of such suppression. This suggests that the increased autoantibody response in RRBC-immunized C57BL/6N mice treated with %MPL%(TM) is not due to the inactivation of suppressor cell activity which, in other studies, was found to be extremely sensitive to %MPL%(TM).

7/3,AB/147 (Item 54 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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02618492 Genuine Article#: LQ741 Number of References: 33 Title: EXPRESSION OF THE C-%MPL% PROTOONCOGENE IN HUMAN HEMATOLOGIC MALIGNANCIES
Author(s): VIGON I; DREYFUS F; MELLE J; VIGUIE F; RIBRAG V; COCAULT L; SOUYRI M; GISSELBRECHT S
Corporate Source: HOP COCHIN,DEPT HEMATOL,27 RUE FAUBOURG/F-75674PARIS 14//FRANCE/; HOP COCHIN,DEPT HEMATOL,27 RUE FAUBOURG/F-75674PARIS 14//FRANCE/; ICGM,INSERM,U363/PARIS//FRANCE/; HOP HOTEL DIEU,CYTOGENET LAB/F-75181 PARIS 04//FRANCE/
Journal: BLOOD, %1993%, V82, N3 (AUG 1), P877-883
ISSN: 0006-4971
Language: ENGLISH Document Type: ARTICLE

7/3,AB/148 (Item 55 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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02568684 Genuine Article#: LM234 Number of References: 43 Title: ANTICARDIOLIPIN %ANTIBODIES% IN OCULOCEREBRAL ISCHEMIA AND MIGRAINE - PREVALENCE AND PROGNOSTIC VALUE
Author(s): HINSE P; SCHULZ A; HAAG F; CARVAJALLIZANO M; THIE A Corporate Source: UNIV HAMBURG,KRANKENHAUS EPPENDORF,DEPT NEUROL/W-2000 HAMBURG 13//GERMANY/; UNIV HAMBURG,KRANKENHAUS EPPENDORF,DEPT MED IMMUNOL/W-2000 HAMBURG 20//GERMANY/
Journal: CEREBROVASCULAR DISEASES, %1993%, V3, N3 (MAY-JUN), P168-173 ISSN: 1015-9770
Language: ENGLISH Document Type: ARTICLE
Abstract: We prospectively studied the prevalence and prognostic value of anticardiolipin %antibodies% (ACLA) in 72 unselected patients with acute or chronic oculocerebral ischaemia (OCI) and 25 patients with migraine. ACLA were measured by ELISA. ACLA titres above 12 GPL-U/ml or 6 %MPL%-U/ml were considered abnormal. Thirty-two of 72 patients with OCI (44%) showed weakly elevated IgG- or IgM-ACLA (i.e. < 20 GPL/%MPL% -U/ml). In 9 patients (12%) ACLA titres were above 20 GPL/%MPL%-U/ml. High ACLA titres in patients with OCI were associated with a significantly increased risk for recurrent cerebral ischaemia. In patients with low ACLA titres, recurrence of ischaemia was no more frequent than in ACLA-negative patients. We suggest that weakly elevated ACLA have no potency for inducing a prothrombotic state and therefore are of no clinical relevance in stroke patients. Serial studies of ACLA titres performed in some patients showed marked fluctuation over time indicating that single determinations should be interpreted with caution. Fifteen of 25 patients with migraine (60%) showed weakly (n = 14, 56%) or highly positive (n = 1, 4%) ACLA titres. Whether ACLA assume a pathogenetic role in migraine remains unknown.

7/3,AB/149 (Item 56 from file: 34)
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02538134 Genuine Article#: LJ534 Number of References: 27 Title: STUDIES OF IMMUNOMODULATING ACTIONS OF CAROTENOIDS .2. ASTAXANTHIN ENHANCES IN-VITRO %ANTIBODY%-PRODUCTION TO T-DEPENDENT ANTIGENS WITHOUT FACILITATING POLYCLONAL B-CELL ACTIVATION
Author(s): JYONOUCHI H; ZHANG L; TOMITA Y Corporate Source: UNIV MINNESOTA,DEPT PEDIAT,DIV IMMUNOL/MINNEAPOLIS//MN/55455; MIYAZAKI UNIV,DEPT AGR,DIV ANIM SCI,ANIM NUTR

&BIOCHEM LAB/MIYAZAKI 88921//JAPAN/
Journal: NUTRITION AND CANCER-AN INTERNATIONAL JOURNAL, %1993%, V19, N3, P 269-280
ISSN: 0163-5581
Language: ENGLISH Document Type: ARTICLE
Abstract: Previously we have shown that astaxanthin, a carotenoid without provitamin A activity, enhances in vitro %antibody% (Ab) production to sheep red blood cells in normal B6 mice. In this study, we further attempted to examine the mechanisms of this enhancing action of carotenoids on specific Ab production in vitro in relation to different antigen (Ag) stimuli, cytokine production, and T- and B-cell interactions in both normal and autoimmune strains of mice. When the actions of carotenoids were tested in normal strains of mice, we found that astaxanthin enhanced in vitro Ab production to T cell-dependent Ag, but not to T-independent Ag, and did not augment total immunoglobulin production. Astaxanthin exerted maximum enhancing actions when it was present at the initial period of Ag priming. This action of astaxanthin was abolished when T cells were depleted from spleen cell suspensions and appeared to require direct interactions between T and B cells. The results also indicated that carotenoids may modulate the production of interferon-T in this assay system. When the actions of carotenoids were tested in autoimmune-prone %MPL% and NZB mice, the enhancing action of astaxanthin on in vitro Ab production was less significant. Furthermore, carotenoids did not potentiate or augment spontaneous Ab and immunoglobulin production by spleen cells in these strains.

Taken together, carotenoids without provitamin A activity may be able to augment in vitro specific Ab production to T cell-dependent Ag partly through affecting the initial stage of Ag presentation without facilitating polyclonal B-cell activation or autoantibody production.

7/3,AB/150 (Item 57 from file: 34)
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02486879 Genuine Article#: LE975 Number of References: 44 Title: CULTURE PH AFFECTS EXPRESSION RATES AND GLYCOSYLATION OF RECOMBINANT MOUSE PLACENTAL-LACTOGEN PROTEINS BY CHINESE-HAMSTER OVARY (CHO) CELLS Author(s): BORYS MC; LINZER DIH; PAPOUTSAKIS ET
Corporate Source: NORTHWESTERN UNIV,DEPT CHEM ENGN/EVANSTON//IL/60208; NORTHWESTERN UNIV,DEPT CHEM ENGN/EVANSTON//IL/60208; NORTHWESTERN UNIV,DEPT BIOCHEM MOLEC BIOL & CELL BIOL/EVANSTON//IL/60208 Journal: BIO-TECHNOLOGY, %1993%, V11, N6 (JUN), P720-724
ISSN: 0733-222X
Language: ENGLISH Document Type: ARTICLE
Abstract: Glycosylation patterns and specific expression rates of the recombinant protein mouse placental lactogen-I (%mPL%-I) by Chinese hamster ovary (CHO) cells varied significantly over the extracellular pH (pH(e)) range of 6.1 to 8.7. The maximum specific %mPL%-I expression rates occurred between pH(e) 7.6 and 8.0. The pH(e) effect on protein expression was confirmed using a different CHO cell expressing the unglycosylated recombinant protein mouse placental lactogen-II (%mPL% -II). Decreases in the extent of glycosylation of %mPL%-I were observed at low (below 6.9) and high (above 8.2) pH(e) values. The pH(e) dependent variations in %mPL%-I accumulation in the supernatant as well as in glycosylation patterns were not the result of enzymatic degradation in the culture medium.

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02255435 Genuine Article#: KP121 Number of References: 30 Title: COMPARISON OF THE INDUCTION OF ENDOTOXIN TOLERANCE IN ENDOTOXEMIA AND PERITONITIS BY MONOPHOSPHORYL LIPID-A AND LIPOPOLYSACCHARIDE Author(s): ASTIZ ME; SAHA DC; BROOKS K; CARPATI CM; RACKOW EC Corporate Source: ST VINCENTS HOSP & MED CTR,NEW YORK MED COLL,DEPT MED,153 W 11TH ST/NEW YORK//NY/10011
Journal: CIRCULATORY SHOCK, %1993%, V39, N3 (MAR), P194-198 ISSN: 0092-6213
Language: ENGLISH Document Type: ARTICLE
Abstract: We compared the induction of endotoxin tolerance with Salmonella minnesota monophosphoryl lipid A (%MPL%), a nontoxic derivative of lipid A, and S. minnesota endotoxin (LPS) in lethal endotoxemia and peritonitis. Lethal endotoxemia was induced by injecting 750 mug/mouse LPS intravenously. Cecal ligation and perforation was used to induce peritonitis. Tumor necrosis factor (TNF) was measured by immunoassay at 2 hr after lethal endotoxin infusion and 24 hr after peritonitis. A dose of 0.1 mug/mouse of %MPL% or LPS significantly reduced endotoxin mortality from 1 00% to 50% and 27%, respectively (P < 0.05). The LD50 for a 0.1 mug dose of %MPL% was 750 mug of LPS and the LD50 for a 0. 1 mug dose of LPS was 1150 mug of endotoxin (P < 0.05). TNF levels decreased linearly when increasing doses of %MPL% and LPS were used to induce tolerance. At higher pretreatment doses of LPS,

survival benefits were attenuated despite the reduction in TNF levels. A 25 mug dose of LPS reduced mortality from peritonitis from 93% to 45% (P < 0.05). Although %MPL% reduced short-term mortality, overall mortality was not significantly reduced despite using large doses of %MPL%. TNF levels peaked at 24 hr and were significantly lower than those following lethal endotoxemia. The induction of endotoxin tolerance by LPS and %MPL% is dose dependent, and LPS is modestly more effective in inducing endotoxin tolerance than %MPL%. Both LPS and %MPL% are significantly less effective in protecting against lethality from peritonitis.

7/3,AB/152 (Item 59 from file: 34)
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00859182 Genuine Article#: FC017 Number of References: 28 Title: ACTIVATION OF MACROPHAGES FROM AGING MICE BY DETOXIFIED LIPID-A
Author(s): CHEN YF; SOLEM L; JOHNSON AG
Corporate Source: UNIV MINNESOTA,DEPT MED MICROBIOL
IMMUNOL/DULUTH//MN/55812; UNIV MINNESOTA,DEPT MED MICROBIOL IMMUNOL/DULUTH//MN/55812
Journal: JOURNAL OF LEUKOCYTE BIOLOGY, %1991%, V49, N4, P416-422 Language: ENGLISH Document Type: ARTICLE
Abstract: A detoxified derivative of endotoxic lipopolysaccharides (LPS), monophosphoryl lipid A (%MPL%), which is capable of inducing nonspecific resistance against several infectious organisms, was tested for its capacity to activate peritoneal macrophages (M-phi) from young and immunodeficient aging BALB/c and C3H/HeN mice. Superoxide generation and hydrogen peroxide release by M-phi from aging mice were elevated following intraperitoneal injection with 25-mu-g of LPS or %MPL%, although they did not reach the peak levels achieved in LPS or %MPL%-treated young mice. Nitroblue tetrazolium reduction (NBT) by peritoneal M-phi from aging C3H/HeN mice treated with %MPL% was higher than that in control aging mice, equalling that from %MPL%-treated young mice. LPS, its toxic counterpart, however, failed to increase NBT reduction in either group. %MPL% enhanced lysozyme activity in M-phi from both aging and young C3H/HeN mice above initial control levels. On the other hand, LPS suppressed lysozyme activity in M-phi from young, but not aging mice. Phagocytosis of Candida albicans by M-phi from BALB/c mice was increased in both groups when stimulated by %MPL%, but not LPS. Similarly, %MPL% enhanced the ability to kill Candida in both aging and young BALB/c mice. This effect was not seen with LPS. Thus ,a detoxified derivative of LPS was found capable of activating the respiratory burst, NBT reduction, elevating lysozyme activity, as well as phagocytosis and killing of Candida in murine peritoneal M-phi from both young and aging mice.

7/3,AB/153 (Item 60 from file: 34)
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00821706 Genuine Article#: EY347 Number of References: 22 Title: THE D-GALACTOSAMINE LOADED MOUSE AND ITS ENHANCED SENSITIVITY TO LIPOPOLYSACCHARIDE AND MONOPHOSPHORYL LIPID-A - A ROLE FOR SUPEROXIDE Author(s): ELLIOTT GT; WELTY D; KUO YD
Corporate Source: RIBI IMMUNOCHEM RES INC,POB 1409/HAMILTON//MT/59840; UNIV MONTANA,DEPT BIOL SCI/MISSOULA//MT/59812
Journal: JOURNAL OF IMMUNOTHERAPY, %1991%, V10, N1, P69-74 Language: ENGLISH Document Type: NOTE
Abstract: Mice are relatively resistant to the lethal effects of endotoxin. Sensitivity to lipopolysacharide (LPS) and monophosphoryl lipid A (%MPL%) can be enhanced by concurrently loading animals with D-galactosamine (D-gal). Significant diurnal variation in susceptibility to lethal toxicity was observed in D-gal loaded mice upon LPS and %MPL% immunostimulant challenge. In mice treated with either %MPL% or %MPL% plus D-gal, at the time of greatest toxic sensitivity, serum TNF levels were significantly higher than was seen in mice treated at a time of low sensitivity. Peritoneal exudate cells (PECs) harvested from mice treated with either D-gal or %MPL% displayed enhanced in vitro superoxide (SO) production. Simultaneous treatment with D-gal and %MPL% led to a synergistic enhancement of SO production above that induced by either xenobiotic alone. Pretreatment with the SO dismutase mimetic Cu(II) (diiso-propyl salicylate)2 significantly protected mice from the lethal toxicity of D-gal-%MPL% challenge. PECs harvested from these same mice failed to display the elevated in vitro SO production reported above. SO elaboration in vivo, presumably by hepatocytes, PECs, and possibly other cells, subsequent to D-gal loading and LPS or %MPL% challenge, appears to play an important role in the lethal toxicity observed. The diurnal variation in toxicity reported in this animal model may result from TNF modulation of SO production in vivo.
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39th Annual Meeting of the American Society for Hematology 9740032 San Diego, CA (USA) 5-9 Dec 1997
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Deng, B.; Banu, N.; Eaton, D.; Wang, J.F.; Cavacini, L.; Avraham, H. 39th Annual Meeting of the American Society for Hematology 9740032 San Diego, CA (USA) 5-9 Dec 1997
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